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&
LIGNARIA
HUNGARICA

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IN FOREST, WOOD
AND ENVIRONMENTAL
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Effects of Meteorological and Site Parameters on the Health Status of Beech (*Fagus sylvatica* L.) Forests in Hungary

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Abstract – The influence of meteorological parameters on the health status of beech (*Fagus sylvatica* L.) was analyzed using long term datasets (1989-2010) collected in 15 sample plots located in Hungary's main beech regions. Leaf loss values were correlated with different meteorological parameters as explanatory variables. Analysis was performed by the CReMIT (Cyclic Reverse Moving Intervals Techniques) method. Weather, stand, and site parameters were also examined with PCA for comparison. Leaf loss levels showed stronger correlations with maximum monthly temperatures than with monthly precipitation sums. The monthly number of summer days and monthly number of hot days displayed a similar correlation to leaf loss as the maximum monthly temperature did. The correlations were regularly stronger and more frequent on more arid sites where the climate is less favorable for beech. Temperature affected leaf loss more than precipitation did. Our results show that beech forests may suffer heavy damage if climate change continues as projected.

beech / *Fagus sylvatica* L. / leaf loss / climate change / CReMIT / Hungary

Kivonat – Meteorológiai és egyes termőhelyi tényezők hatása a bükk (*Fagus sylvatica* L.) egészségi állapotára Magyarországon. A meteorológiai tényezők bükkösökre gyakorolt hatásait 15 mintaterületen gyűjtött hosszútávú (1989-2000) adatsorokon vizsgáltuk. A lombvesztés értékét különböző meteorológiai tényezőkkel korreláltattuk, a CReMIT (Cyclic Reverse Moving Intervals Techniques) mozgó időablakos módszer segítségével. Ezután az időjárási, termőhelyi és erdőállomány-jellemzőket is bevonva PCA analízist is végrehajtottunk. A lombvesztés erősebb kapcsolatot mutatott a havi maximum hőmérséklettel, mint a havi csapadék-összeggel. A havi nyári napok és hőségnapok összegei a maximum-hőmérséklethez hasonló összefüggéseket mutattak. A korrelációk erőssége nagyobb volt a szárazabb klímájú, bükknek kevésbé alkalmas mintaterületeken. Eredményeink alátámasztják, hogy a jövőben a bükkösök súlyos károknak lehetnek kitéve, ha a klímaváltozás az előrejelzett forgatókönyvek szerint alakul majd.

bükk / *Fagus sylvatica* L. / lombvesztés / klímaváltozás / CReMIT / Magyarország

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1 INTRODUCTION

Although the ratio of European beech (*Fagus sylvatica* L.) forests is relatively low in Hungary (112,603 ha; 6.0% of total forested area – NFK 2020), the species still possesses ecological and economic importance. Hungarian beech forests are situated on low to mid-mountain elevations, and on extrazonal occurrences in lower, hilly areas. Due to its distinct climatic needs, beech is considered a forest-climate indicator species in Hungary. Optimal montane/submontane sites with preferable climate for beech have a restricted extent in Hungary; hence, beech forests often grow in suboptimal sites.

Beech has significant precipitation needs and weak drought tolerance (Arend et al. 2016), which makes it vulnerable to climate change (Geßler et al. 2007). This is especially true in conditions and sites where beech forests thrive in Hungary today. Nonetheless, climate change appears to have the opposite effect in other north-eastern European provenances where suitable areas for beech are expanding (Augustaitis et al. 2015).

Climate change scenarios project worsening conditions for beech forests in Southern and Eastern Europe, particularly in the Carpathian Basin (Berki et al. 2007). Some researchers suggest beech may even disappear from Hungary altogether in the next century (Mátyás et al. 2010, Führer et al. 2011). Central and Southern European beech stands have suffered incremental reduction during drought and warm years since 1980 (Jump et al. 2006, Zimmermann et al. 2015, Giagli et al. 2016). A reduction in suitable habitat for beech due to climate change is also projected for the Iberian Peninsula (del Río et al. 2018).

Hungarian forests already face direct or indirect damage related to extreme weather conditions, particularly drought damage (Csóka et al. 2009, Lakatos – Molnár 2009, Rasztovits et al. 2014, Janik et al. 2016). In Hungary, beech is close to its xeric limits, which makes it more susceptible to climate change.

As in many other European countries, severe beech declines were recorded at several locations in Hungary in the late 1980s. This the FRI (Forest Research Institute) to launch the beech health monitoring plot network in 1989 (Koltay 2004). The initiative was based on the experiences of the ICP-Forests network (Michel – Siedling 2014), and its aim was to closely monitor the health trends of Hungarian beech stands and, subsequently, analyze the collected data. In this paper we processed the dataset on beech monitoring plots, and used statistical methods to identify correlations between meteorological, forest site, forest stand, and leaf-loss parameters.

Our starting hypothesis predicted direct positive correlations between temperature-related parameters and leaf loss, and negative correlations between precipitation and leaf loss. We also expected delayed and/or cumulative effects of weather from previous years.

2 MATERIALS AND METHODS

2.1 Location and main parameters of the sample plots

Altogether, 32 beech monitoring plots have been established in Hungary since 1989. Individual sample trees were marked and numbered on the monitoring plots. The stands in which the plots are located were managed by state forest companies, according to normal management plans. All the plots were established with 100 sample trees; over time, the number of trees gradually decreased due to mortality, intermediate cutting, etc. In our present analysis, we selected 15 plots that fulfilled the criteria listed below:

- The time series was at least 15 years in duration,
- The number of sample trees in 2010 was at least 50,
- The decrease of the number of sample trees during the examined period did not exceed 30%.

2.2 Site and stand parameters

The ages of the monitored stands varies between 64 and 135 years. *Table 1* provides other basic information about the monitored stands. The sample trees were surveyed once a year, in early September. NARIC FRI researchers completed the assessments. The visual survey was executed according to ICP methodology, and the surveyors regularly participated in international calibration workshop events (ICP Forests.net). For this study, we used the assessed leaf-loss values of the sample trees within 5% accuracy, but we scored several other parameters as well. Leaf loss is an important health indicator, and it is also comparable to other results. Only sample trees in Kraft classes 1 (outstanding/dominant) and 2 (codominant) (Kraft 1884) were included in the analysis since the trees in other social classes are too strongly influenced by competition for light.

Forest stand data involving our plots were obtained from the “Hungarian forest inventory”, from the National Land Center, Department of Forestry. This database contains detailed information (including site and stand parameters) about the location of the monitoring plots in the forest compartments.

Table 1. Basic data about the 15 selected monitoring plots included in the study

Forest subcompartment	Coordinates	First year	N (pcs) (first year)	N (pcs) (2010)	Age (2010)	Altitude (m)
Bőszénfa 12E	N46 15.727 E17 47.470	1992	100	98	128	300
Felsőtárkány 140D	N48 02.394 E20 28.986	1994	100	98	101	600
Felsőtárkány 55A	N48 01.515 E20 24.938	1994	100	99	95	700
Füzér 86F	N48 34.248 E21 25.785	1992	97	81	86	700
Füzér 86G	N48 34.123 E21 25.707	1992	99	80	81	700
Gyöngyössolymos 41B	N47 52.918 E19 57.480	1992	100	98	108	700
Kislőd 4B	N47 11.512 E17 38.402	1996	90	61	117	500
Kőszeg 43H	N47 22.103 E16 27.902	1992	76	64	133	800
Nagyhuta 10C	N48 28.157 E21 25.580	1995	89	66	89	400
Orfű 21B	N46 07.610 E18 10.292	1992	69	62	62	500
Répáshuta 11C	N48 03.288 E20 32.060	1994	100	100	83	700
Répáshuta 12G	N48 03.135 E20 32.290	1994	99	97	78	800
Szentpéterfölde 20A	N46 36.055 E16 44.973	1989	68	42	119	300
Ugod 31A	N47 17.534 E17 39.765	1992	120	84	101	500
Zselickislak 8E	N46 15.617 E17 48.380	1995	100	99	126	400

2.3 Meteorological parameters

Data was provided by the Hungarian Meteorological Service as a 10 km x 10 km grid interpolated data set from standard weather station measurements by the MISH method (Szentimrey et al. 2005). We used the following monthly weather parameters from the data series: maximum monthly temperature; monthly precipitation sum; monthly number of hot days, monthly number of summer days. We chose monthly maximum temperature instead of average temperature because extreme weather events are generally more damaging to tree health. Defined meteorologically, hot days are those in which the maximum daily temperature reaches/exceeds 30 °C. Summer days are those in which the daily maximum temperature reaches/exceeds 25 °C. The meteorological dataset was available between 1961 and 2010.

2.4 Statistics

Leaf loss data collected in a long-term monitoring network was correlated with different meteorological indices using CReMIT (Cyclic Reverse Moving Intervals Techniques). This is a special moving-window based method implementing moving averages and moving interval techniques (Pödör et al. 2014). The method makes it possible to systematically increase the number of used dependent or independent variables by creating new, derived time series from the basic dataset in a systematic way. Next, linear regression was used to examine the relationships between the derived meteorological parameters as independent variables, and the health status (indicated by leaf loss) as dependent variables. The significance of the results was examined by Student's t-test. To see the effects of the two previous years' weather on forest health, we examined correlations dating back 36 months. In our CReMIT analysis, the time windows did not extend beyond five months. We examined the year of the leaf loss assessment, the previous year's damage, and the leaf loss assessments from two years ago.

With temperature, precipitation, and number of summer days as parameters, we chose May, June, July, August, and September as the time windows for the three examined years. We omitted winter precipitation amounts since we decided to present results that were comparable to all weather indices.

Only the time windows from June to September were feasible for the number of hot days; thus, the length of the input time series was not exactly even. Nevertheless, through our selection of method and sample plots, we endeavored to ensure the differences would not significantly impact the results.

Forest health data was correlated with all of the derived meteorological parameters. This data was available for all the sample trees, so it was possible to stratify the results into Kraft-classes (1 – predominant, 2 – dominant, 3 – codominant and partially dominated, 4 – overtopped) (Kraft 1884, in Assmann 1968).

The CReMIT method created all of the continuous time series from the previous three years with the above-mentioned different window sizes ranging from one to five months.

Once the CReMIT results were achieved, the sample plots were examined according to forest site, meteorological, and stand parameters to determine if these results were similar to the CReMIT results. Principal Component Analysis (PCA) was chosen to perform this examination, mainly because the method can also be used if the parameters are not (or not fully) independent (Pearson 1901). Forest site parameters were included: in the case of forest soil, topsoil layer thickness and slope are crucial as both affect soil water dynamics. Exposure (0 = north slope; 180 = south slope) and altitude define the mesoclimate.

Forest stand parameters were also used in PCA: species mixture, canopy closure, and social position (Kraft classes). Stem diameter and stand height can be complex variables due to site conditions; hence, these variables were excluded from our examinations. *Table 3* contains the 14 examined environmental parameters.

Basic database management was performed with Libreoffice and Microsoft Office software. We also used R software environment and PAST v.2.17c (Hammer et al. 2001) software for statistical analysis.

3 RESULTS

The significant CReMIT results were rather diverse. On some occasions, neighbouring sample plots with extremely similar site conditions (and with the same interpolated meteorological datasets) surprisingly exhibited different responses. Only results with 90, 95, and 99% significance levels of individual time windows were summarized in *Table 2*.

Positive correlations were expected between “maximum monthly temperature” and leaf loss. Four plots gave consistently strong relationships; as a result, the expected correlations appeared. Unexpected negative correlation values emerged for plots located at higher elevations. These results occurred sporadically in six plots, but with one plot (Orfű 21B), the negative correlation was remarkable between the given year’s leaf loss and also with the temperature values from two years prior. In Kraft class 2, the results were usually similar, with the exception of the Kislőd plot.

As expected, “summer days” gave results similar to “maximum monthly temperature”. Although the number of plots with significant correlations were lower, the significance levels were usually stronger. In five plots, unexpected negative results occurred, which were similar to the maximum temperature results.

The number of “hot days” were obviously fewer than the number of “summer days”, but the results were similar. The significant correlations were even stronger, but fewer in number. Negative correlation values occurred only in one plot, in two time windows, in the Kraft class 2.

In the case of “monthly precipitation sum”, negative correlation values were presumed. The expected negative correlations occurred in eight plots; five of these had remarkably strong significant correlations. Unexpectedly, positive correlations occurred in five plots. Orfű 21B had a remarkable and unexpected correlation with the values from two years before the actual leaf loss values. The Kraft 2 class also shows similar but weaker correlations. The main results of the CReMIT analysis are summarized in *Table 2*.

Table 2. Evaluation of the CReMIT results by scoring the strength according to the frequency of significant (above 90%) time windows (1, 2, 3) and direction (“–” = negative correlation; “+” = positive correlation) of correlations between leaf loss and weather parameters in a given year (0) and the previous two years (–1; –2)

Parameter	Maximum monthly temperature			Monthly precipitation sum			Summer days			Hot days		
	0	–1	–2	0	–1	–2	0	–1	–2	0	–1	–2
Location/Year	0	–1	–2	0	–1	–2	0	–1	–2	0	–1	–2
Böszénfa 12E	3	0	0	–2	0	0	3	0	0	3	0	0
Felsőtárkány 140D	0	0	2	0	0	0	–1	0	1	0	0	0
Felsőtárkány 55A	0	0	0	0	0	0	0	0	1	0	0	0
Füzér 86F	2	0	0	–3	0	–1	3	0	0	0	0	0
Füzér 86G	2	0	0	–2	0	–2	3	0	0	0	0	0
Gyöngyössolymos 41B	0	0	0	0	–1	0	0	0	0	0	2	0
Kislőd 4B	2	1	0	–2	0	0	2	1	0	3	1	0
Kőszeg 43H	1	0	–1	0	0	0	2	0	0	3	0	0
Nagyhuta 10C	–1	–2	–1	–2	–2	0	–2	–2	0	0	0	–1
Orfű 21B	3	0	–2	0	0	2	3	0	–1	3	0	0
Répáshuta 11C	0	–1	0	0	2	0	0	0	0	1	0	0
Répáshuta 12G	0	–1	0	0	1	0	0	0	0	3	0	0
Szentpéterfölde 20A	1	1	–1	0	1	1	0	0	–1	1	1	0
Ugod 31A	0	0	3	2	1	0	0	–1	3	0	0	1
Zselickislak 8E	3	0	0	–2	0	0	3	1	0	3	0	0

Furthermore, CReMIT results (based on leaf-loss data series) were compared with forest site data collected via PCA analysis, which was performed in 15 (meteorological, site-dependent, and stand-dependent parameters all together) variables. This resulted in four new variables (4 Axes) that together account for 85% of the variation in forest site variables of the

sample plots (Table 3). Meteorological parameters were represented in the PCA analysis by their averages for all sample plots and for the whole examination period. Axis 1 is strongly positively correlated with the topsoil layer thickness and temperature variables, and negatively correlated with altitude. This relationship is obvious since average temperature normally increases as altitude decreases. Similarly, on lower altitudes, topsoil layers are usually thicker. The second component (Axis 2) is negatively correlated with slope, crown closure, and the proportion of Kraft class 1 and 2; it is also positively correlated with precipitation. As can be observed in the variance values of the components, temperature, and topsoil layer thickness are the main parameters. It is also clear that temperature is more relevant than precipitation sum. These support the results derived from time series analysis (CReMIT). Similar to weather, temperature has the most influence on health status at the long term level.

The third component (Axis 3) is determined by mixing ratio, and the fourth (Axis 4) contains exposure/facing and the age of trees. Only the mixing ratio determines the third component.

Table 3. Eigenvalues, proportion of variance and environmental variable loadings for the first 4 principal components in PCA

Axes	Axis 1	Axis 2	Axis 3	Axis 4
<i>Statistical parameters</i>				
Eigenvalue	6.0	2.6	1.8	1.4
Explained variance	43.0%	18.9%	13.0%	10.2%
Cumulated variance	43.0%	61.9%	74.9%	85.1%
<i>Environmental variables</i>				
Altitude	-0.809	-0.070	0.264	0.161
Exposure/Facing	0.324	0.210	-0.510	-0.516
Slope	-0.441	-0.539	-0.447	0.460
Topsoil layer thickness	0.904	0.059	0.250	0.064
Mixture ratio	-0.138	0.357	0.873	-0.094
Age	0.425	0.328	-0.081	0.638
Crown closure	-0.346	-0.636	0.378	-0.483
Average temperature	0.937	0.159	0.068	0.166
Maximum temperature	0.955	-0.206	-0.013	0.104
Sum of hot-days	0.924	-0.068	0.241	-0.151
Sum of summer-days	0.974	-0.126	0.047	-0.047
Precipitation sum	-0.122	0.934	-0.022	-0.014
Precipitation sum between May-Aug.	-0.559	0.581	0.213	0.208
Proportion of Kraft class 1/2	0.088	-0.601	0.447	0.412

The PCA detected three groups among the sample plots (Figure 1). These groups are colored red, yellow, and green. The red group contains the sample plots that had the strongest correlation between leaf loss and weather parameters according to CReMIT. The other plots in PCA also show an order similar to the results of CReMIT.

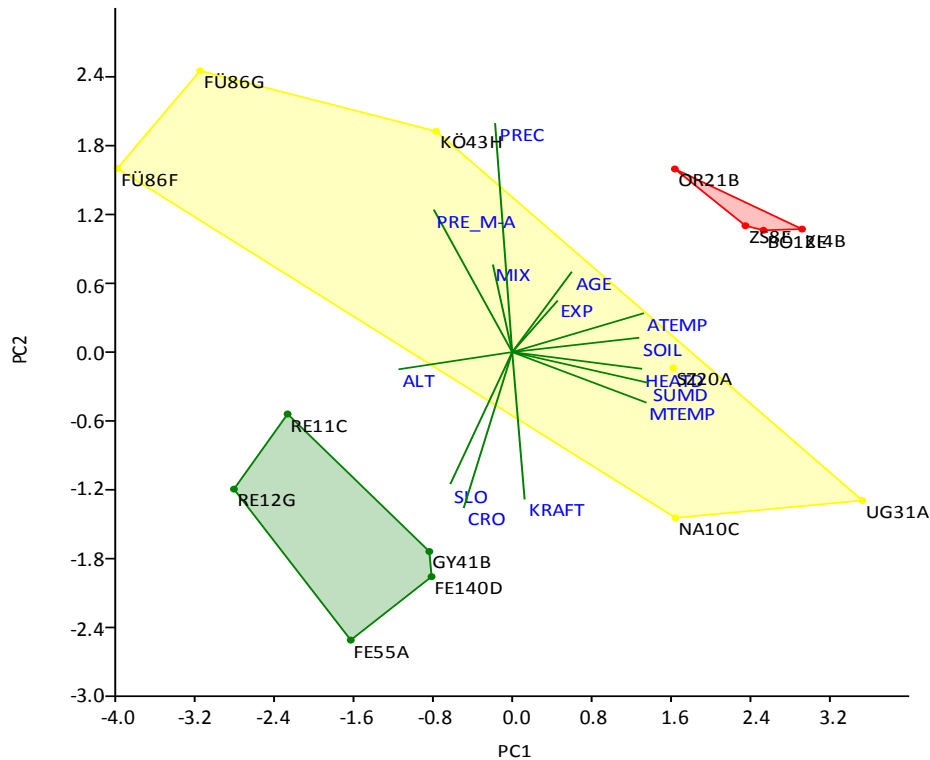


Figure 1. Scatter biplot of PCA. The diagram displays both the loadings (correlations between the original environmental variables and the components) as labelled vectors and the component scores of survey plots as labels and colored points. Survey plots with similar parameters are grouped together and linked with colored convex hulls. Plot names are abbreviated with the first 2 and the last 3 characters.

4. DISCUSSION

In this paper we processed a previously gathered dataset on beech health, and searched for statistically detectable correlations with weather indices (in an available meteorological dataset). Our methods successfully detected known connections and demonstrated that these methods are serviceable even in extremely complex systems. That temperature affects leaf loss much more than precipitation sum is an important result from a forest protection perspective. If higher mean temperatures do occur (as they have in recent years), neither the same amount nor a slightly higher amount of precipitation will prevent beech trees from declining. We also could statistically support that beech forests in less suitable climatic conditions are more susceptible to weather extremities.

More specifically, most of the results supported our preliminary hypotheses: temperature and precipitation-type weather parameters have a consequential effect on beech health. Both of the used statistical methods supported these presumptions.

As we assumed, the CReMIT method resulted in a relatively low number of significant correlations. Since forest ecosystems are extremely complex, we could not expect simple linear correlations, as mentioned by Berki et al. (2014). Detecting statistical patterns in forests is often difficult (Granier et al. 1999). Even so, significant results could be detected with weather parameters two years before the actual leaf loss. Time windows in the damage year gave the strongest significant results. Additionally, as was the case in our work, studies examining the weather dependence of beech growth with different running window statistical methods also

detected correlations with various drought indexes (Manninger et al. 2011, Garamszegi – Kern 2014).

Monthly maximum temperature showed frequent and remarkable positive correlations with leaf loss, which is similar to conclusions many researchers have reached regarding average temperature (Siedling 2007, Zimmermann et al. 2015, Popa et al. 2017). In our analysis, this effect could even be detected with temperature values two years before the actual leaf loss. The number of hot days had a high significance in some cases, but the lower occurrence made patterns undetectable.

In our results, the effect of monthly precipitation sums on beech health was weaker than the effect of monthly maximum temperature. As expected, precipitation sums showed mostly negative correlations with leaf loss in our results. Precipitation sum values from previous years had some significant correlations on some time windows, as expected, but the actual year had the greatest effect (in particular sample plots most time windows had significant correlations) on the health status. These findings coincide with results of previous European studies, namely that higher amounts of precipitation have a positive effect on beech health (Meier – Lauschner 2008a, Seletković et al. 2009, Delaporte et al. 2016).

Although our input datasets were not optional (we had to use the available data from our sample plots), the weaker effects of precipitation compared to temperature and the unexpected correlations may raise a potential methodological issue: Using non-locally measured data (10x10 km grid interpolation in our case) may not be reliable enough to reveal the real correlations because the spatial variation of precipitation is very high (Manninger 2017). This may, at least partly, explain the controversial correlations. For every meteorological index, we detected that nearly the same plots had stronger correlations, while other plots had no responses or weak significant responses. The four plots showing the strongest responses kept this feature in almost every parameter and in both Kraft classes.

Sample plots situated in less favorable sites showed consistently stronger correlations with weather parameters. In fact, the plots are all situated in areas where the climate had become suboptimal for beech stands. According to Gálos and Führer (2018), this even applies to beech stands established from 1971-2010. The weather extremes in suitable sites affect health status less intensively. Although our plots are not situated on an ideal altitude for beech, many sample plots may remain relatively healthy. On the lower edge of the climate belt suitable for beech, the health statuses were generally worse.

It is known that many forest stand and forest site parameters impact beech health; for example: stands with higher canopy closure are less susceptible to health problems than those with low canopy closure (Csóka et al. 2009; Bošela et al. 2016). Mixed stands also appear to be more resistant and resilient than artificially grown monoculture beech forests (Mölder – Leuschner 2014, Metz et al. 2016). Nevertheless, seedlings and young trees in mixed forests are still vulnerable to extreme droughts (Lübbe et al. 2015).

PCA detected site and stand parameters, which allowed for the establishment of three identifiable groups among the plots. We could conclude that the differences in the correlations between forest health and weather indices (CReMIT results) in the different sample plots show the same variances, which can be obtained by the PCA examination of climate, forest stand, and site data. This fact implies that differences are caused by these climatic, site, and stand parameters. The importance ranking of these parameters on beech health in our results is monthly maximum temperature, altitude, topsoil layer thickness, monthly precipitation sum, tree social class, canopy closure, slope, species mixture, stand age, and exposure. The patterns of PCA and CReMIT results among the sample plots are almost identical, so the statistical variances caused by leaf loss and site parameters are also almost identical. This implies that the weather effect on beech health is influenced by forest stand and site parameters. Additionally,

it is influenced in the manner PCA calculated (Table 3). This also suggests that beech health risks can be projected based on forest site and stand data.

These results support earlier findings (Neiryneck – Roskams 1999, Stribley – Ashmore 2002, Siedling 2007, Seletković et al. 2009). According to Potočić et al. (2008), during years without weather extremes, the correlation between weather and leaf loss is not particularly remarkable, but a strong connection can be detected in extremely droughty years when the correlation between leaf loss and temperature appears much stronger. Popa et al. (2017) also found different responses of beech health to the weather parameters. In areas where beech occurs naturally, temperature increase has a positive effect on the health status. Wherever beech grows outside this area, precipitation has a moderately positive effect on health, while temperature increase has a negative effect. On the upper edge of the beech area (in high mountains), temperature had a clearly positive effect. On optimal forest sites for beech, both temperature and precipitation had little effect on tree health (Popa et al. 2017). These findings are the logical results of the process of forest acclimatization to weather: the variability of environmental parameters in the long term established a natural range of a certain species that have low impact influence in the “core area”. On the margins, these parameters either open a new frontier for the species, or reduce the area of the species.

Tree-ring analyses provided similar results and correlations between ring width and early summer temperatures than with late summer precipitations in Central Europe. With lower altitudes – which regularly means worsening climate for beech – the effects become stronger (Kolář et al. 2017).

There are many ways to improve the research of weather and forest health relationships. Permanent “on the spot” meteorological parameter measurements would be a major step forward. Applying health indicators other than leaf loss (i.e. measuring photosynthetic activity, sap flow, etc.) could also be a way forward.

Some weather parameters – those not included in our study – could also modify health responses. Cloudy weather (occurring often in mountain valleys) can greatly decrease leaf transpiration (Rozas et al. 2015). Air humidity can also be an important factor. Furthermore, the quantity of soil nitrogen can exacerbate the effects of drought (Dziedek et al. 2016). Measuring these parameters in the future would be extremely useful for understanding beech health responses.

Individual tree level differences in sensitivity and response are also considerable since intraspecific/intrapopulation diversity (i.e. phenological cycle) is typical in all populations and biological processes (Delpierre et al. 2017).

It would be very useful to forecast beech damage – possibly similarly to Italian researchers who found a fungal pathogen’s presence or absence (*Biscogniauxia nummularia*) to be a suitable sign for indicating drought damage in Mediterranean areas (Luchi 2015).

Some opinions suggest that the southern provenances should be more adapted and, therefore, more resistant to a warmer and drier climate (Cavin – Jump 2016, Horváth – Mátyás 2016). Others believe this type of adaptation potential is not strong enough in the case of beech (Knutzen et al. 2015), or that genetic diversity affects the shoot and the leaf growth more than the root growth, which is crucial from a drought tolerance perspective (Meier – Leuschner 2008b).

The predictive site choice of a given tree species (or the tree species choice for a given site) is increasingly important. The climate tolerance of different tree species must be carefully considered since this significantly influences the stability of future forests (Hlásny et al. 2017).

Moreover, if climate change proceeds in the manner some projections predict, more frequent and severe damage events will occur. These events will be far more connected to weather conditions than they currently are (Gálos – Führer 2018). Clearly, a proactive silviculture involving mixed

beech stands, increased structural diversity, and high canopy closure (avoiding large cutting areas) may considerably reduce the risks imposed by the changing climate.

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Antioxidant Capacity and Tentative Identification of Polyphenolic Compounds of Cones of Selected Coniferous Species

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Abstract – The cones of coniferous species are a waste biomass byproduct that can be potentially utilized for a variety of purposes. One of the many application fields is the extraction of bioactive materials, particularly antioxidant polyphenols. Scientific literature on the antioxidant content of coniferous cones at different ripening stages is limited. In this study, we conducted a comparative analysis of the antioxidant content of selected taxa that are either common in Hungary or that have not yet been investigated in the scientific literature in any great detail (*Cedrus atlantica*, *Larix decidua*, *Picea abies*, *Pinus mugo*, *Pinus nigra*, *Pinus sylvestris*, *Pinus wallichiana*, *Tsuga canadensis*, *Tsuga heterophylla*, *Chamaecyparis lawsoniana*, *Taxodium distichum*, *Thuja occidentalis*, *Metasequoia glyptostroboides*, *Thuja orientalis*, *Cryptomeria japonica*, *Cunninghamia lanceolata*). A comparison of green, mature and opened cones was performed for the assigned taxa. Folin-Ciocalteu total polyphenol content (TPC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to assess the antioxidant contents. Overall antioxidant power was determined by a scoring system that combined the three assay results. In general, best values were found for green cones, followed by mature, and opened cones for each taxon. *Tsuga canadensis*, *Metasequoia glyptostroboides*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Thuja orientalis* and *Picea abies* all contained high amounts of antioxidants in both green and mature cones and attained the highest scores. High-performance liquid chromatographic/tandem mass spectrometric profiling of the cone polyphenols was also completed for selected samples. Results provide a basis for future bioactivity testing of these samples.

coniferous species / cones / antioxidants / HPLC-MS/MS

Kivonat – Tülevelű taxonok tobozainak antioxidáns kapacitása és polifenolos vegyületeinek vizsgálata. A tülevelű fajok tobozai olyan hulladék biomasszát képviselnek, melyeket többféle célra is lehetne használni. Az egyik ilyen felhasználási terület a bioaktív anyagok, például antioxidáns polifenolok kinyerése. A tobozérés különböző fenofázisaiban az antioxidáns tartalomra vonatkozó szakirodalmi adatok hiányosak. Jelen cikkben olyan taxonok vizsgálatát végeztük el, melyek vagy Magyarországon gyakoriak, vagy még nem történt meg a vizsgálatuk (*Cedrus atlantica*, *Larix decidua*, *Picea abies*, *Pinus mugo*, *Pinus nigra*, *Pinus sylvestris*, *Pinus wallichiana*, *Tsuga canadensis*, *Tsuga heterophylla*, *Chamaecyparis lawsoniana*, *Taxodium distichum*, *Thuja occidentalis*, *Metasequoia glyptostroboides*, *Thuja orientalis*, *Cryptomeria japonica*, *Cunninghamia lanceolata*). Elvégeztük a zöld, érett és lehullott tobozok összehasonlító vizsgálatát az összes polifenol tartalom (Folin-Ciocalteu), a FRAP (ferric reducing antioxidant power) és a DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidáns kapacitás meghatározási módszerek segítségével. Az összesített antioxidáns kapacitás kiértékelése a

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három módszer egyesítésével, egy pontrendszer segítségével történt meg. Összességében a legnagyobb antioxidáns kapacitást a zöld tobozokra mértük, a legalacsonyabbat a lehullott tobozokra mindegyik taxon esetében. A legmagasabb pontszámot a *Tsuga canadensis*, *Metasequoia glyptostroboides*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Thuja orientalis* és *Picea abies* zöld és értett tobozai kapták. A kiválasztott minták esetében elvégeztük a polifenol készlet profilozását nagyhatékonyságú folyadékkromatográfiás/tandem tömegspektrometriás eljárással. Az eredmények alapját képezhetik ezen minták bioaktivitás-vizsgálatának.

tűlevelű fajok / toboz / antioxidánsok / HPLC-MS/MS

1 INTRODUCTION

Forestry, logging and timber production wastes (e.g. leaves, wood bark, cones, etc.) can be a rich source of antioxidant compounds (Dedrie et al. 2015, Bouras et al. 2016) with potential utilization fields including the production of healthcare-related products (Packer et al. 1999, Dzialo et al. 2016, Watson et al. 2018), natural food preservatives and ingredients (Coté et al. 2011, Gyawali – Ibrahim 2014, Kobus-Cisowska et al. 2014, Frydman et al. 2005), natural growth bioregulators (Popa et al. 2008, Vyvyan 2002) as well as silver nanoparticles (Fahimirada et al. 2019, Rolim et al. 2019) to name but a few.

As waste biomass basic materials, cones represent a biomass exclusively born by coniferous trees and shrubs belonging to one of the over 615 living species (Auders – Spicer 2012). Conifers bear “seed-cones” and “pollen-cones” out of which the female seed-cones are simply referred to as “cones”; these were the exclusive subject of the present study.

The primary use of forest tree cones has been seed extraction for the production of forestry propagation material. In the Mediterranean region the edible seeds of stone pine cones (*Pinus pinea* L.) are one of the most important tree nuts (Kemerli-Kalbaran – Ozdemir 2019). The empty cones are usually burned (Aniszewska – Bereza 2014) in an uncompressed state or can be converted to briquettes (Gendek et al. 2018). The cones *Juniperus* spp. have traditionally been used for flavouring purposes (Lesjak et al. 2011), while the cone extracts and essential oils of *Pinus*, *Thuja*, and *Cedrus* spp. have been used by traditional medicine for various beneficial (e.g. anti-inflammatory, antioxidant, antiseptic, antifungal, antimicrobial, analgesic etc.) health effects (Watanabe et al. 1995, Lesjak et al. 2011, Süntar et al. 2012, Djouahri et al. 2014). The cone extracts of *Pinus parviflora* Siebold et Zucc. were shown to be very powerful against HIV and influenza viruses (Nagata et al. 1990) and were also shown to possess significant antimutagenic and anticancer effects (Nagasawa et al. 1992). The cone and essential oil extracts of *Metasequoia glyptostroboides* (Bajpai et al. 2014), *Juniperus sibirica* Burgsdorf. (Lesjak et al., 2011), *Tetraclinis articulata* (Vahl) Mast. (Djouahri et al. 2014), *Cupressus sempervirens* var. *pyramidalis* (L.) (Tumen et al. 2012) and of *Pinus* spp. (Süntar et al. 2012, Bradley et al. 2014, Tümen et al. 2018, Wang et al. 2019) were recently shown to have significant beneficial effects on human health. The latest results indicate that pine cone and pine cone extracts can be used for their various useful properties, e.g. being a source as dietary fibre (Kartal – Ozturk 2016), or starting materials for the production of coagulants (Hussain et al. 2019) and adsorbents (Kupeta et al. 2018, Mtshatsheni et al. 2019).

Despite the listed results, the literature lacks systematic research of the antioxidant composition of cones and the assessment of their role as a source of natural antioxidants. Moreover, sample collection times in the presented examples – more specifically, the phenophase of cone maturity – have rarely been documented in the literature. Recently Hofmann et al. (2020) concluded a systematic research using optimized extraction conditions and multiassay evaluation for the assessment of the antioxidant content of coniferous cones while respecting the phenophase of cone maturity; however, this study included only 6 taxa.

The aim of the present research was to extend previous studies (Hofmann et al. 2020) by investigating altogether 16 taxa including Atlas cedar (*Cedrus atlantica* Endl.), European larch (*Larix decidua* Mill.), Norway spruce (*Picea abies* H. Karst.), mountain pine (*Pinus mugo* Turra), black pine (*Pinus nigra* J.F. Arnold), Scots pine (*Pinus sylvestris* L.), Himalayan pine (*Pinus wallichiana* A. B. Jacks.), eastern hemlock (*Tsuga canadensis* (L.) Carrière), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Lawson cypress (*Chamaecyparis lawsoniana* (A. Murray) Parl.), bald cypress (*Taxodium distichum* (L.) Rich.), northern white-cedar (*Thuja occidentalis* L.), dawn redwood (*Metasequoia glyptostroboides* Hu and W. C. Cheng), Chinese arborvitae (*Thuja orientalis* L.), Japanese cedar (*Cryptomeria japonica* (L.f.) D. Don) and China fir (*Cunninghamia lanceolata* (Lamb.) Hook).

Antioxidant properties were assayed by the Folin-Ciocalteu total polyphenol content (TPC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. The evaluation of the overall antioxidant power was accomplished by a scoring system, which combined the results of the TPC, FRAP, and DPPH methods. In this manner a comprehensive evaluation of the results between various samples with potentially different antioxidant compositions was achieved.

The polyphenol profile of most relevant samples with the highest antioxidant potential was also investigated using high-performance liquid chromatography/multistage mass spectrometry (HPLC-MS/MS) in order to identify the structure of major antioxidant compounds, primarily polyphenols.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Double distilled water was prepared for the extractions using conventional distillation equipment. LCMS grade acetonitrile, and acetone was obtained from VWR International (Budapest, Hungary). Gallic acid, ascorbic acid, DPPH, 2,4,6-tripyridyl-S-triazine (TPTZ), iron(III)-chloride, acetic acid, sodium acetate, hydrochloric acid, and sodium carbonate were obtained from Sigma-Aldrich (Budapest, Hungary). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany).

2.2 Sample collection and extraction

Sample collection occurred at the Botanical Garden of the University of Sopron in Sopron, Hungary between July-October 2019. Three ripening stages were sampled: green cones (collected in July when cones are green, yet nearly at their full size at the final year of maturation), mature cones (collected in August/September when the cones turned brown in colour and scales began to open) and opened cones (taken in September/October, at a fully opened state having released their seeds and found on trees or to the ground). One healthy individual of each taxon was sampled by collecting a minimum of 10 cones from different parts of the crown at each sampling occasion. Cone samples were put into sealed plastic bags and stored at -20°C until processing. Prior to extraction, samples were thawed and ground. Ultrasonic extraction was performed using an Elma Transsonic T570 ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) as follows: 0.45 g ground sample was homogenized with 45 ml acetone:water 80:20 v/v in a 50 ml centrifuge tube and sonicated for 3 x 10 min as described by Hofmann et al. (2020). One extraction was conducted for each sample.

2.3 Determination of antioxidant properties

TPC, FRAP, and DPPH measurements were run in triplicates using of a Hitachi U-1500 type spectrophotometer (Hitachi Ltd., Tokyo, Japan).

2.3.1 Total polyphenol content (TPC)

TPC determination was completed by applying the Folin-Ciocalteu assay (Singleton – Rossi 1965) using gallic acid as the standard: 0.5 ml extract solution was mixed with 2.5 ml 10-fold diluted Folin-Ciocalteu reagent. After 1 min, 2 ml 0.7 M Na₂CO₃ solution was added and the reaction mixture was heated for 5 min in a 50 °C water bath. Reaction was stopped by cooling to room temperature in a cold water bath. Solution absorbance was measured at 760 nm. The results were expressed as mg equivalents of gallic acid/g dry bark units (mg GAE/g d.w.).

2.3.2 FRAP antioxidant capacity

The method described by Benzie – Strain (1996) was applied for the measurement of the FRAP antioxidant capacity at 593 nm using ascorbic acid as a standard. FRAP reagent was prepared as follows: 25 ml of 10 mM TPTZ solution (aqueous with 84 µl cc. HCl) was mixed with 250 ml of acetate buffer (300 mM, pH 3.6) and 25 mL of 20 mM aqueous FeCl₃ solution. Fifty µl sample was mixed with 1500 µl FRAP reagent in glass test tubes at ambient temperature and after 5 min reaction time absorbance was measured. Results were given in mg equivalents of ascorbic acid/g dry weight (mg AAE/g dw.).

2.3.3 DPPH antioxidant capacity

The slightly modified method of Sharma – Bhat (2009) was used for running the DPPH assay as follows: 2090 µl unbuffered methanol was mixed with 900 µl 2×10⁻⁴ M methanolic DPPH solution and 10 µl extract. After 30 min incubation at room temperature in the dark, the decrease in absorbance was determined at 515 nm. Results were calculated in IC₅₀ (50% inhibition concentration) values in µg extractives/ml assay (µg/ml) units, representing the amount of extractives which will react with 50% of the added DPPH• radicals in the total assay volume (3 ml).

2.4 HPLC-MS/MS analyses

Separation of the cone extracts of Norway spruce and eastern hemlock was achieved using a Shimadzu LC-20 type high-performance liquid chromatograph coupled with a Shimadzu SPD-M20A type diode array detector (PDA) (Shimadzu Corporation, Kyoto, Japan) and an AB Sciex 3200 QTrap triple quadrupole/linear ion trap mass spectrometric (MS) detector (AB Sciex, Framingham, USA). A Phenomenex Synergy Fusion-RP 80A, 250 mm x 4.6 mm, 4µm column was used for the separation with a Phenomenex SecurityGuard ULTRA LC type guard column (Phenomenex Inc., Torrance, USA) at 40°C. The injection volume was 15 µl. The binary gradient of A (H₂O + 0.1% HCOOH) and B (CH₃CN + 0.1% HCOOH) solvents was run with 1.2 ml/min flow-rate using the following time gradient: 3% B (0-4 min), 6% B (10 min), 20% B (34 min), 57% B (73 min), 100% B (90-98 min), 3% B (99-106 min). The PDA detector signal (250-380 nm) was recorded to monitor separation of peaks. Negative electrospray ionization mode was used for the MS detector by allowing 0.6 mL/min flow to enter the MS ion source using a split valve. Polyphenols were identified with the Information Dependent Analysis (IDA) scanning function of the mass spectrometer using a survey (Q1) scan between 150-1300 m/z and respective dependent (Q3) product ion scans between 80-1300 m/z. Ion source settings were as follows: spray voltage: -4500 V, source temperature: 500°C, curtain gas (N₂) pressure: 40 psi, spray gas (N₂) pressure: 30 psi, drying gas (N₂) pressure: 30 psi. Chromatographic data were acquired and evaluated using the Analyst 1.6.3 software. Mass

spectra evaluation and compound identification was achieved using the RIKEN tandem mass spectral database (Sawada et al. 2012), via the scientific data found in the literature and by the use of fragmentation rules (McLafferty – Tureček 1993).

2.5 Statistics

In order to compare the respective antioxidant capacities of the extracts, ANOVA analysis was run using Statistica 11 (StatSoft Inc., Tulsa, USA) software with the Tukey HSD method.

3 RESULTS AND DISCUSSION

3.1 Evaluation of the TPC, FRAP and DPPH results

Table 1 includes the TPC, FRAP, and DPPH data of the samples indicating statistical comparison (ANOVA) for the 10 best results within each method. In all of the investigated taxa, the highest TPC was measured in green cone samples, followed by mature and opened cone samples. Overall the highest TPC was determined in the green cones of eastern hemlock (157.25 ± 9.98 mg GAE/g dw.), Lawson cypress (131.68 ± 4.35 mg GAE/g dw.), Japanese cedar (131.74 ± 3.00 mg GAE/g dw.) and dawn redwood (113.60 ± 4.81 mg GAE/g dw.). Respecting mature and opened cones highest TPC values were determined for dawn redwood (mature: 91.25 ± 3.69 mg GAE/g dw., opened: 60.16 ± 8.23 mg GAE/g dw.), Chinese arborvitae (mature: 81.22 ± 5.30 mg GAE/g dw., opened: 68.88 ± 4.91 mg GAE/g dw.), Japanese cedar (mature: 74.18 ± 2.09 mg GAE/g dw., opened: 57.41 ± 2.93 mg GAE/g dw.) and Norway spruce (mature: 64.64 ± 2.68 mg GAE/g dw., opened: 46.39 ± 3.54 mg GAE/g dw.).

According to literature data, Hofmann et al. (2020) determined high TPC levels for Norway spruce and eastern hemlock green cone samples. Horiba et al. (2016) found 84.9 ± 3.3 mg GAE/g dw TPC in Japanese cedar cones (without seeds), which is comparable to the present results.

The overall highest TPC, determined for eastern hemlock green cones (157.25 ± 9.98 mg GAE/g dw.) was surprisingly higher than that of the related taxon, western hemlock (89.16 ± 5.51 mg GAE/g dw.). In fact, Hernes – Hedges (2004) reported the tannin content of western hemlock cones to be 3.13 wt.%; however, the authors did not document either the phenophase of cone maturity or the month of the sample collection. Hernes – Hedges (2004) also found that the bark and green needles contained more tannins compared to cones, yet did not investigate the amount of other types of polyphenols.

The limitation of the Folin-Ciocalteu assay (Singleton – Rossi 1965) is that it is known to interfere with other types of antioxidants (Prior et al. 2005, Everette et al. 2010). In fact, the TPC method is considered one of the >100 different assays currently used for the determination of antioxidant capacity and radical scavenging ability (Cornelli 2009). None of these assays is individually able to measure the total antioxidant power of all compounds in plant extracts. Therefore, the use of multiple assays to estimate the “overall” antioxidant potential of complex extracts is recommended (Ghiselli et al. 2000). The present study used the FRAP and the DPPH methods to provide further results on the antioxidant power of the samples.

Table 1. TPC^1 , $FRAP^2$, and $DPPH^3$ antioxidant capacity of the cones (mean $\pm$ standard deviation). Different superscript letters indicate significant differences at $p < 0.05$ (TPC, FRAP, DPPH) between the samples with the 10 best values

	TPC (mg GAE/g dw.)			FRAP (mg AAE/g dw.)			DPPH IC ₅₀ (μ g extractives/ml)		
	Green	Mature	Opened	Green	Mature	Opened	Green	Mature	Opened
Atlas cedar	88.41 $\pm$ 1.68	14.96 $\pm$ 2.24	7.46 $\pm$ 0.26	62.08 $\pm$ 3.13 ^a	4.48 $\pm$ 0.11	3.37 $\pm$ 0.10	21.44 $\pm$ 2.94	88.82 $\pm$ 12.86	56.92 $\pm$ 15.87
European larch	83.44 $\pm$ 4.27	25.98 $\pm$ 0.94	17.60 $\pm$ 2.15	55.96 $\pm$ 0.93	14.18 $\pm$ 0.83	4.09 $\pm$ 0.17	9.07 $\pm$ 1.39	12.53 $\pm$ 0.38	28.21 $\pm$ 6.84
Norway spruce	105.58 $\pm$ 7.92 ^{ab}	64.64 $\pm$ 2.68	46.39 $\pm$ 3.54	72.02 $\pm$ 8.76 ^{ab}	50.19 $\pm$ 2.08	28.35 $\pm$ 3.37	10.75 $\pm$ 0.32	9.38 $\pm$ 1.14	8.57 $\pm$ 0.17 ^{ab}
Mountain pine	95.76 $\pm$ 9.48 ^a	22.33 $\pm$ 3.31	15.96 $\pm$ 1.10	60.06 $\pm$ 2.77	9.34 $\pm$ 0.07	7.25 $\pm$ 0.19	7.87 $\pm$ 0.31 ^{abc}	27.83 $\pm$ 3.73	18.86 $\pm$ 0.14
Black pine	89.22 $\pm$ 4.79	19.70 $\pm$ 3.36	7.08 $\pm$ 0.34	58.21 $\pm$ 2.34	9.55 $\pm$ 0.52	4.50 $\pm$ 0.17	15.33 $\pm$ 1.39	45.90 $\pm$ 2.69	62.32 $\pm$ 1.90
Scots pine	46.30 $\pm$ 1.81	18.99 $\pm$ 1.44	13.19 $\pm$ 1.53	33.42 $\pm$ 3.12	9.41 $\pm$ 0.32	7.26 $\pm$ 0.14	72.40 $\pm$ 21.26	29.32 $\pm$ 1.10	22.88 $\pm$ 0.54
Himalayan pine	62.52 $\pm$ 5.09	17.76 $\pm$ 1.35	8.18 $\pm$ 0.97	38.84 $\pm$ 0.69	8.33 $\pm$ 0.56	3.85 $\pm$ 0.21	25.72 $\pm$ 3.50	54.76 $\pm$ 14.54	72.58 $\pm$ 7.23
Eastern hemlock	157.25 $\pm$ 9.98 ^d	56.13 $\pm$ 4.07	10.57 $\pm$ 1.69	100.11 $\pm$ 0.40 ^e	46.57 $\pm$ 1.02	5.94 $\pm$ 0.25	7.83 $\pm$ 0.29 ^{abc}	11.37 $\pm$ 0.67	17.74 $\pm$ 1.01
Western hemlock	89.16 $\pm$ 5.51	30.77 $\pm$ 2.22	10.01 $\pm$ 1.77	59.11 $\pm$ 1.73	31.03 $\pm$ 1.55	4.53 $\pm$ 0.09	11.16 $\pm$ 1.37	15.52 $\pm$ 0.84	40.44 $\pm$ 17.94
Lawson cypress	131.68 $\pm$ 4.35 ^c	20.61 $\pm$ 2.27	16.21 $\pm$ 2.11	89.42 $\pm$ 6.82 ^{cde}	9.18 $\pm$ 0.12	8.36 $\pm$ 0.13	7.23 $\pm$ 0.41 ^{bc}	22.46 $\pm$ 1.72	30.50 $\pm$ 6.72
Bald cypress	70.99 $\pm$ 4.49	52.20 $\pm$ 1.86	29.53 $\pm$ 3.96	57.34 $\pm$ 1.28	49.69 $\pm$ 5.07	42.42 $\pm$ 3.29	8.45 $\pm$ 0.74 ^{ab}	13.17 $\pm$ 2.13	13.42 $\pm$ 0.60
Northern white-cedar	93.71 $\pm$ 5.47 ^a	39.96 $\pm$ 2.59	31.38 $\pm$ 2.57	76.46 $\pm$ 3.44 ^{abc}	49.81 $\pm$ 0.11	18.54 $\pm$ 0.83	9.93 $\pm$ 0.62	9.21 $\pm$ 0.30	8.13 $\pm$ 0.55 ^{ab}
Dawn redwood	113.60 $\pm$ 4.81 ^b	91.25 $\pm$ 3.69 ^a	60.16 $\pm$ 8.23	129.16 $\pm$ 3.01 ^f	147.00 $\pm$ 6.83 ^g	61.43 $\pm$ 3.51	6.22 $\pm$ 0.42 ^c	4.42 $\pm$ 0.07 ^d	7.15 $\pm$ 0.87 ^{bc}
Chinese arborvitae	106.67 $\pm$ 2.76 ^{ab}	81.22 $\pm$ 5.30	68.88 $\pm$ 4.91	78.49 $\pm$ 1.55 ^{bcd}	93.12 $\pm$ 4.84 ^{de}	31.60 $\pm$ 2.02	9.56 $\pm$ 0.50	15.76 $\pm$ 0.45	17.27 $\pm$ 7.71
Japanese cedar	131.74 $\pm$ 3.00 ^c	74.18 $\pm$ 2.09	57.41 $\pm$ 2.93	60.87 $\pm$ 5.21	41.04 $\pm$ 2.08	24.16 $\pm$ 0.86	10.13 $\pm$ 0.76	10.55 $\pm$ 1.40	17.51 $\pm$ 0.56
China fir	92.24 $\pm$ 1.57 ^a	36.36 $\pm$ 2.29	35.94 $\pm$ 1.33	67.99 $\pm$ 8.88 ^{ab}	37.20 $\pm$ 2.68	20.65 $\pm$ 1.44	9.03 $\pm$ 1.19 ^a	13.79 $\pm$ 0.46	11.14 $\pm$ 0.45

1: Total polyphenol content

2: Ferric reducing antioxidant power

3: 2,2-diphenyl-1-picrylhydrazyl

Regarding FRAP results, green cone samples showed the best results in general. The only opposite tendency was observed with dawn redwood and Chinese arborvitae, where mature cones (D.r.: 147.00 ± 6.83 mg AAE/g dw., C.a: 93.12 ± 4.84 mg AAE/g dw.) had superior FRAP values compared to green cone results (D.r.: 129.16 ± 3.01 mg AAE/g dw., C.a: 78.49 ± 1.55 mg AAE/g dw.) showing excellent FRAP. Overall the best FRAP was determined for the green cones and opened cones of previous two taxa and for the green cones of eastern hemlock (100.11 ± 0.40 mg AAE/g dw.). According to Lesjak et al. (2011, 2014), the FRAP of *Juniperus* spp. cones varies between 3.61 ± 0.03 mg AAE/g dw. (*Juniperus macrocarpa* Sibth. et Sm.) to 35.26 ± 1.12 mg AAE/g dw. (*Juniperus sibirica* Burgsdorf.), which also indicates that big differences between related taxa can exist, as is the case with eastern (100.11 ± 0.40 mg AAE/g dw.) and western hemlock (59.11 ± 1.73 mg AAE/g dw.) cones in the present study.

The DPPH radical scavenging activity was determined using the IC₅₀ value (50% inhibition concentration), with low IC₅₀ indicating high antioxidant capacities. The DPPH results also showed the general decreasing tendency of the order green > mature > opened cones within a given taxon. The best results were obtained for the mature (4.42 ± 0.07 µg/ml) and green (6.22 ± 0.42 µg/ml) cones of dawn redwood, and for green cones of Lawson cypress (7.23 ± 0.41 µg/ml) and eastern hemlock (7.83 ± 0.29 µg/ml). In fact, the excellent DPPH activity (Bajpai et al. 2009, 2017) and bioactivity (Bajpai et al. 2007, 2009, Yoon et al. 2011) of dawn redwood cone extracts has already been reported in scientific literature.

The TPC, FRAP, and DPPH data makes it apparent that all of the three assays indicated different orders for the best results, which was attributed to the different compositions of the extracts as well as to the different working principle and selectivity of the assays (Apak et al. 2007, Müller et al. 2011).

In order to obtain a comprehensive measure of the overall antioxidant efficiency of the cone extracts and to consider the different selectivity of methods, the summarized evaluation of results of the three different methods was implemented.

3.2 Combined evaluation of the TPC, FRAP and DPPH results

Combined evaluation of the TPC, FRAP and DPPH was achieved using a scoring system (Hofmann et al. 2020) with the following calculation: For the TPC and FRAP results, 0 points were assigned to the weakest values and 1 to the best values within each assay, using linear approximation for the other values. In the case of DPPH assay, opposite scoring was used (lowest IC₅₀ value, score: 1; the highest IC₅₀, score: 0). The respective scores of TPC, FRAP and DPPH were then summarized for each sample to estimate the measure of the overall antioxidant efficiency (Table 2).

Regarding the sum of scores, the highest scores – those with the best overall antioxidant power – were determined in the green cones of eastern hemlock (2.63), dawn redwood (2.56), Lawson cypress (2.40), Japanese cedar (2.16), Chinese arborvitae (2.13) and Norway spruce (2.06) and for the mature cones of dawn redwood (2.56). Interestingly eastern hemlock contained much higher overall antioxidant power compared to related western hemlock for green, mature and opened cone samples, showing big differences between respective samples; this discrepancy requires further research to determine an explanation.

Of these taxa, the bioactivity, antioxidant activity, or uses of their cone extracts have already been reported in the literature for Lawson cypress (Smith et al., 2007, Kilinc et al. 2015), dawn redwood (Bajpai et al. 2007, 2009, 2014, 2017, Yoon et al. 2011), Japanese cedar (Horiba et al. 2016) and Chinese arborvitae (Yogesh – Ali 2014).

However, no data on the polyphenolic composition and bioactivity of Norway spruce and eastern hemlock cone extracts exists in the scientific literature. Norway spruce is one of the most widespread coniferous tree species in Europe, possessing significant ecological, industrial

and economic significance (Meloni et al. 2007, Lamedica et al. 2011). Eastern hemlock is an ecologically important foundation species in forests of eastern North America (Clark et al. 2012) with a natural range extending from northern Georgia and Alabama to southern Canada and westward into the central Great Lakes states (McWilliams – Schmidt 2000). Information on molecular cone extract composition will provide a basis for the future research on the role these compounds play in possible bioactivity effects. Hence, the remainder of this article will focus on the identification of cone extractives, especially polyphenolic compounds found in the green cone tissues of Norway spruce and eastern hemlock.

Table 2. Normalized values (scores) of the TPC¹, FRAP², and DPPH IC₅₀³ values and the sum of scores for each sample representing the combined antioxidant values.

	TPC ¹			FRAP ²			DPPH IC ₅₀ ³			Sum of scores		
	Gr.	Mat.	Op.	Gr.	Mat.	Op.	Gr.	Mat.	Op.	Gr.	Mat.	Op.
Atlas cedar	0.54	0.05	0.00	0.41	0.01	0.00	0.80	0.00	0.38	1.75	0.06	0.38
European larch	0.51	0.13	0.07	0.37	0.08	0.00	0.94	0.90	0.72	1.82	1.10	0.79
Norway spruce	0.66	0.38	0.26	0.48	0.33	0.17	0.93	0.94	0.95	2.06	1.65	1.39
Mountain pine	0.59	0.10	0.06	0.39	0.04	0.03	0.96	0.72	0.83	1.94	0.87	0.92
Black pine	0.55	0.08	0.00	0.38	0.04	0.01	0.87	0.51	0.31	1.80	0.64	0.32
Scots pine	0.26	0.08	0.04	0.21	0.04	0.03	0.19	0.71	0.78	0.66	0.83	0.85
Himalayan pine	0.37	0.07	0.01	0.25	0.03	0.00	0.75	0.40	0.19	1.36	0.51	0.20
Eastern hemlock	1.00	0.33	0.02	0.67	0.30	0.02	0.96	0.92	0.84	2.63	1.54	0.88
Lawson cypress	0.83	0.09	0.06	0.60	0.04	0.03	0.97	0.79	0.69	2.40	0.92	0.79
Bald cypress	0.43	0.30	0.15	0.38	0.32	0.27	0.95	0.90	0.89	1.75	1.52	1.31
Northern white-cedar	0.58	0.22	0.16	0.51	0.32	0.11	0.93	0.94	0.96	2.02	1.49	1.22
Dawn redwood	0.71	0.56	0.35	0.88	1.00	0.40	0.98	1.00	0.97	2.56	2.56	1.75
Chinese arborvitae	0.66	0.49	0.41	0.52	0.62	0.20	0.94	0.87	0.85	2.13	1.98	1.46
Japanese cedar	0.83	0.45	0.34	0.40	0.26	0.14	0.93	0.93	0.84	2.16	1.64	1.32
China fir	0.57	0.19	0.19	0.45	0.24	0.12	0.95	0.89	0.92	1.96	1.32	1.23
Western hemlock	0.55	0.16	0.02	0.39	0.19	0.01	0.92	0.87	0.57	1.85	1.22	0.60

1: Total polyphenol content

2: Ferric reducing antioxidant power

3: 2,2-diphenyl-1-picrylhydrazyl

Gr.: green cones, Mat.: mature cones, Op.: opened cones

3.3 HPLC-MS/MS analyses

The identification of the molecular structure of the extractives in the cone extract solutions of Norway spruce and eastern hemlock has been accomplished using high-performance liquid chromatography/tandem mass spectrometry. *Figure 1.* depicts the HPLC chromatograms and *Table 3* includes the major compounds found in the extracts.

Altogether 82 compounds have been described and tentatively identified by tandem mass spectrometric fragmentation (MS/MS) data. The composition of the green cones of the two taxa is different, with both including low amounts of (+)-catechin (3), (–)-epicatechin (7), and procyanidin B dimers (1, 2, 4). Extracts included a large number of coumaric acid derivatives and flavonoid glycosides, yet not all of the compounds were found in both samples.

Quercetin-*O*-hexosides (18, 19) and taxifolin-*O*-hexosides (12,13) were found in both taxa; however, the pentose conjugate of quercetin (21) was only indicated in eastern hemlock. Interestingly, isorhamnetin-*O*-hexosides (27, 28) were only found in Norway spruce. The most abundant class of flavonoid conjugates were the kaempferol derivatives (mostly glycosides) with a total count of 10 compounds. Out of these compounds only kaempferol-*O*-hexoside (25), kaempferol-*O*-rutoside (37) and kaempferol-rhamnose-hexose-rhamnose (50) were detected in the green cones of both taxa. The *O*-rutoside (24), *O*-pentoside (29, 30, 31), *O*-rhamnoside (33), acetyl-hexoside (34), and an unknown derivative (46) of kaempferol were exclusively detected in eastern hemlock. Regarding flavonoid glycosides, the presence of acylated kaempferol conjugates (e.g. 34) are especially interesting as these types of compounds were shown to have excellent antioxidant properties and to contribute significantly to antibacterial effects of plant extracts (Mellou et al. 2005), which highlights the importance in finding matrices with high content of acylated flavonols (García-Villalba et al. 2017).

The presence of coumaric acid as part of the compounds was evidenced by the simultaneous presence of the 163, 145, and 119 *m/z* ions in the MS/MS spectra of the compounds corresponding to the $[M-H]^-$, $[M-H_2O-H]^-$ and $[M-CO_2-H]^-$ fragment ions, with M representing coumaric acid molecule. The structure of coumaric acid derivatives are often left unidentified using ion trap or triple quadrupole mass spectrometers (Spínola et al. 2016, Llorent-Martínez et al. 2019), as the MSⁿ mass spectra are only suitable to indicate characteristic fragments and losses during the fragmentation process of the molecules, justifying the simultaneous presence of the coumaric acid fragments at 119, 145 and 163 *m/z*. A more precise and informative analysis of the structure of these compounds could be conducted in the future with the use of TOF (time-of-flight) mass spectrometry by determining accurate mass of the compounds (Vilhena et al. 2020). Coumaric acid derivatives 47, 48, 49, 59, and 66 were only indicated in Norway spruce, while compounds 55, 60, and 65 were found exclusively in eastern hemlock and compound 51 in the green cone extracts of both taxa.

Piceatannol isomers (15, 16) and their *O*-hexoside conjugates (possibly astringin isomers, 10, 11) were evidenced from spruce samples only.

Chlorogenic acid isomers (5, 6) were only found in eastern hemlock. Other compounds were left unidentified only with MS/MS data for future identification of their structure.

According to Table 3 and comparing peak heights in Figure 1, the most abundant compounds in the green cone extract of Norway spruce were astringin isomers (10, 11), unidentified compounds 8, 58, 68, 69, 70 and coumaric acid derivative 51, while in eastern hemlock they were chlorogenic acid isomers 5, 6, kaempferol-rhamnose-hexose-rhamnose 50, and unidentified compounds 68, 69, 70, and 79.

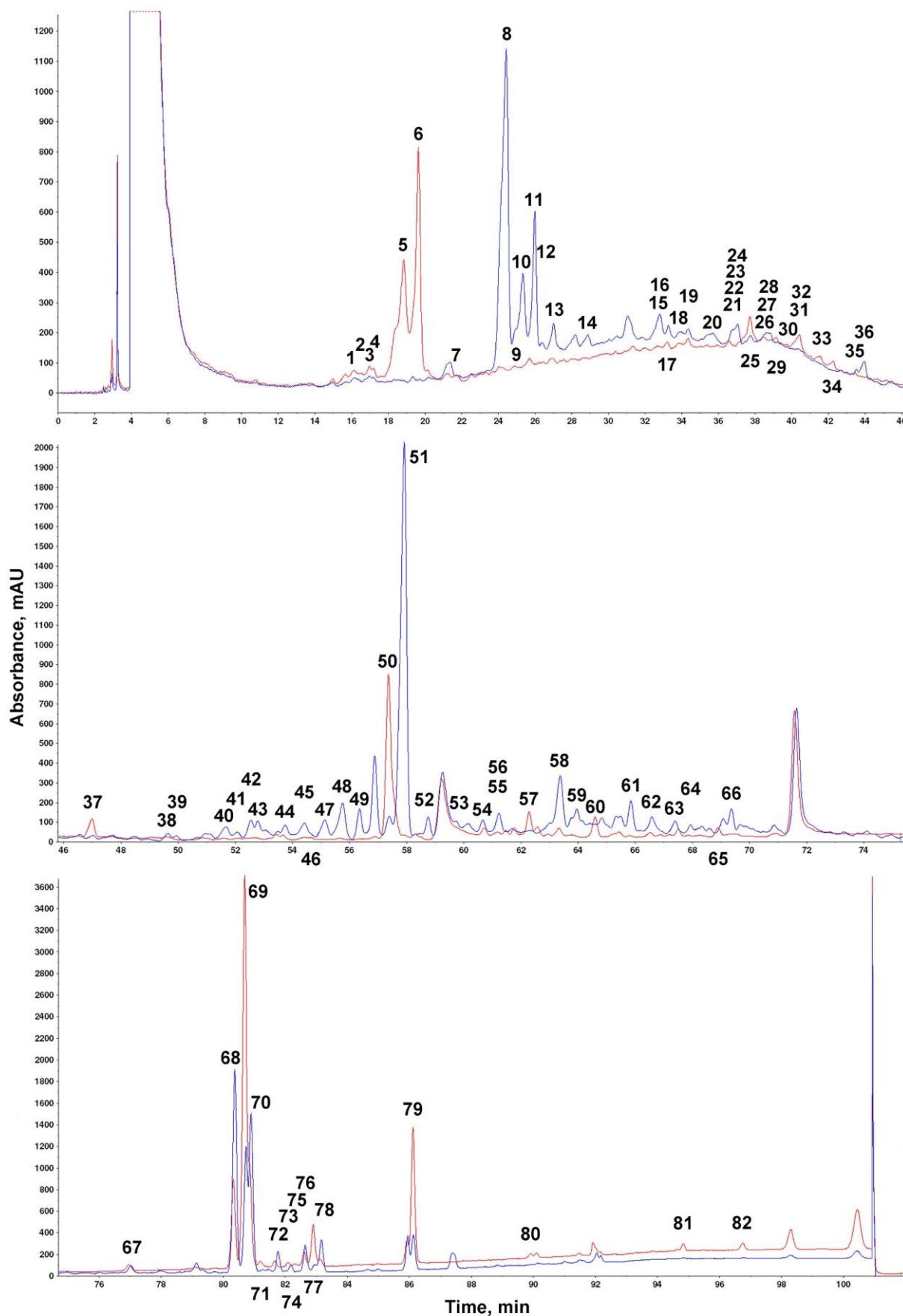


Figure 1. The PDA (250-380 nm) chromatogram of the green cone extracts of Norway spruce (blue) and eastern hemlock (red).

Table 3. Tentative chromatographic/mass spectrometric identification of the polyphenols in the green cones of Norway spruce (S) and eastern hemlock (H)

Peak	t_r (min)	Compound	S	H	[M-H] ⁻ m/z	MS/MS m/z
1	15.8	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
2	16.2	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
3	17.0	(+)-Catechin	x	x	289	245, 203, 125, 123, 109
4	17.2	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
5	18.9	Chlorogenic acid isomer		x	353	191, 179, 161, 135
6	19.7	Chlorogenic acid isomer		x	353	191, 179, 161, 135
7	21.7	(-)-Epicatechin	x	x	289	245, 203, 125, 123, 109
8	24.0	Unidentified	x		no ion	no negative ions
9	25.0	Unidentified	x		no ion	no negative ions
10	25.3	Piceatannol-O-hexoside (astringin)	x		405	243, 225, 201
11	26.0	Piceatannol-O-hexoside (astringin)	x		405	243, 225, 201
12	26.3	Taxifolin-O-hexoside	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
13	27.1	Taxifolin-O-hexoside	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
14	29.0	Unidentified	x		285	241, 217, 199
15	32.6	Piceatannol	x		243	225, 201, 175, 174
16	32.8	Piceatannol	x		243	225, 201, 175, 174
17	33.3	Unidentified	x		257	241, 211,
18	33.9	Quercetin-O-hexoside	x	x	463	301, 300, 271, 255, 179
19	34.4	Quercetin-O-hexoside	x	x	463	301, 300, 271, 255, 179
20	35.4	Unidentified	x		359	341, 311, 297, 282, 195, 163, 145
21	36.6	Quercetin-O-pentoside		x	433	301, 300, 271, 255, 243, 179
22	36.8	Unidentified	x		373	358, 313, 305
23	37.0	Unidentified	x		359	341, 311, 297, 282, 195, 163, 145
24	37.2	Kaempferol-O-rutinoside		x	593	447, 285, 284, 255, 227
25	37.7	Kaempferol-O-hexoside	x	x	447	285, 284, 255, 227
26	38.2	Unidentified-O-hexoside		x	431	268, 269
27	38.6	Isorhamnetin-O-hexoside	x		477	315, 314, 300, 299, 271
28	38.9	Isorhamnetin-O-hexoside	x		477	315, 314, 300, 299, 271
29	39.2	Kaempferol-O-pentoside		x	417	285, 284, 255, 227
30	39.8	Kaempferol-O-pentoside		x	417	285, 284, 255, 227
31	40.4	Kaempferol-O-pentoside		x	417	285, 284, 255, 227
32	40.5	Unidentified-O-hexoside	x	x	447	315, 285, 217, 199
33	41.6	Kaempferol-O-rhamnoside		x	431	285, 284, 255, 277
34	42.2	Kaempferol-acetyl-hexoside		x	489	429, 285, 284, 255, 227
35	43.6	Unidentified	x	x	351	333, 315, 275, 251
36	43.9	Unidentified	x		291	245, 175
37	47.0	Kaempferol-O-rutinoside	x	x	593	447, 285, 284, 255, 227
38	49.8	Unidentified	x	x	351	333, 315, 275, 251
39	50.0	Unidentified	x		367	349, 321, 247
40	51.7	Unidentified	x		377	331
41	52.0	Unidentified	x		331	313, 273, 241, 185
42	52.6	Unidentified	x		349	331, 287, 251, 244, 207, 189, 163
43	52.8	Unidentified	x		405	375, 337, 327, 275
44	53.7	Unidentified	x		401	333, 315, 257
45	54.4	Unidentified	x		521	179, 162, 146, 135
46	54.7	Kaempferol derivative		x	635	285, 284
47	55.1	Coumaric acid derivative	x		445	427, 397, 349, 277, 251, 163, 145, 119
48	55.8	Coumaric acid derivative	x		475	457, 427, 281, 163, 145, 119
49	56.4	Coumaric acid derivative	x		505	487, 457, 311, 163, 145, 119
50	57.4	Kaempferol-rhamn.-hex.-rhamn.	x	x	739	593, 453, 285, 284, 255, 229

Table 3 cont. Tentative chromatographic/mass spectrometric identification of the polyphenols in the green cones of Norway spruce (S) and eastern hemlock (H)

Peak	t_r (min)	Compound	S	H	[M-H] ⁻ m/z	MS/MS m/z
51	58.0	Coumaric acid derivative	x	x	505	491, 477, 342, 327, 312, 177, 163, 119
52	58.8	Unidentified	x		535	520, 491, 341, 326, 193, 179, 134
53	59.7	Unidentified	x	x	445	417, 399, 315
54	60.7	Unidentified	x	x	401	333, 315, 289, 245
55	61.1	Coumaric acid derivative		x	549	489, 353, 311, 163, 145, 119
56	61.2	Unidentified	x		349	331, 289, 245
57	62.1	Unidentified	x	x	399	367, 331, 299
58	63.4	Unidentified	x	x	385	317, 299, 253
59	64.0	Coumaric acid derivative	x		667	521, 403, 323, 163, 145, 119
60	64.6	Coumaric acid derivative		x	653	638, 507, 489, 353, 329, 177, 163, 145, 119
61	66.0	Unidentified	x		383	355, 315, 297
62	66.6	Unidentified	x		383	315, 299, 269
63	67.4	Unidentified	x		471	425, 403, 353, 325, 285
64	68.0	Unidentified	x	x	381	313, 269
65	68.9	Coumaric acid derivative		x	651	487, 472, 341, 326, 266, 163, 145, 119
66	69.4	Coumaric acid derivative	x		649	441, 426, 411, 321, 291, 253, 163, 145, 119
67	77.0	Unidentified	x	x	429	381, 299, 265
68	80.4	Unidentified	x	x	687	657, 301
69	80.7	Unidentified	x	x	397	301
70	80.9	Unidentified	x	x	431	401, 383, 301
71	81.2	Unidentified		x	469	425, 410, 384, 367, 339, 285
72	81.7	Unidentified	x		455	409, 391, 387, 355, 287
73	82.1	Unidentified		x	957	467, 423, 381
74	82.2	Unidentified	x		455	409, 391, 387, 355, 287
75	82.4	Unidentified		x	935	467, 424, 382, 265
76	82.6	Unidentified	x	x	721	417, 335, 317
77	82.9	Unidentified		x	467	449, 423, 408, 382, 338
78	83.1	Unidentified	x	x	633	333, 317, 315, 299
79	86.1	Unidentified		x	635	591, 333, 317, 301, 271
80	89.9	Unidentified		x	769	725, 467, 301
81	94.8	Unidentified		x	501	486
82	96.7	Unidentified		x	529	514

rhamn.: rhamnose; hex.: hexose

4 CONCLUSIONS

The present study compared and evaluated the antioxidant capacity of the cone extracts of 16 selected coniferous taxa. The overall antioxidant power was determined by a scoring system that combined the results of the three antioxidant assays used in the study. The best antioxidant properties were determined for green cones, followed by mature and opened cones for each taxon. The highest scores were found for *Tsuga canadensis*, *Metasequoia glyptostroboides*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Thuja orientalis* and *Picea abies*, which contained high amounts of antioxidants in both green and mature cones. The high-performance liquid chromatographic/tandem mass spectrometric profiling of the green cone extractives of *Picea abies* and *Tsuga canadensis* was carried out and overall 82 compounds have been tentatively identified from these samples for the first time, including kaempferol-, taxifolin-, quercetin- and isorhamnetin-O-glycosides, coumaric acid derivatives, chlorogenic acids,

piceatannol and its conjugates, and flavan-3-ol compounds. Presented chromatographic/mass spectrometric data on the polyphenolic composition of the green cone extracts contributes to the determination of the structure of unidentified compounds and to the research on the role of extractives in determining the bioactivity of cone extracts. To enhance practical use of this study's results, future research will focus on the antibacterial and antifungal properties of the investigated cone extracts with the highest antioxidant capacity.

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Effect of Non-polar Extractable Substances on Soils and on Vegetation Cover from old Environmental Burdens

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Abstract – This case study focuses on the assessment of the effect of soil pollution by gudrons disposed in landfills. Waste products are acid tars, called "gudron" in the Slovakian terminology. Gudrons are waste products resulting from sulphonation technologies used in oil processing. In the Slovak Republic, gudron landfills are risk localities and are classified as old environmental burdens. Non-polar extractable substances (NES) as well as the activity of soil cellulase and basal soil respiration in soil samples taken from four different distances from the pollution sources were analysed. The effect of landfills on vegetation was assessed by recording the number and cover of plants on the sampling points. Long-term and gradual gudron contamination of the surrounding areas from both landfills is evident and has been proven by monitored NES concentrations. The pollution progress was predicted by the use of logistical function (based on the NES indicator) due to the increasing distance from the sources of pollution. Comparison of these two areas showed markedly higher oil substances pollution in the soil samples taken from the surroundings of the landfill Predajna 2. Determined content of NES did not meet the criteria of permissible concentration in soil samples, not even at a distance of 150 m ($< 0.1 \text{ mg kg}^{-1}$ in compliance with the Law No. 220/2004 Coll.). When determining basal soil respiration, the production of CO_2 corresponded with oil pollution determined by the NES indicator. High concentrations of NES hinder enzymatic cellulase activity. The decomposition of cellulose occurs only at lower concentrations of NES. It is possible to range the soils of lower NES concentrations (soils taken from the distances of 70 m and 150 m from Predajna 1; 110 m and 150 m from Predajna 2) among the soils with weak or middle soil cellulase activity. This indicates that microbial activity was detected in the soil samples, and the values of this microbial activity were higher due to a decrease of inhibitors caused by oil pollution. That total surface vegetation cover increases as distance from the landfills increases indicated the validity of these facts.

soil cellulase activity / basal soil respiration / non-polar extractable substances / residues from oil processing / oil pollution

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Kivonat – Felhagyott hulladéklerakók környezeti terhelésének vizsgálata az apoláris kioldható anyagok talajra és a növényzetre gyakorolt hatásán keresztül. Kutatásunk során a hulladéklerakókban elhelyezett gudron okozta talaj szennyezés hatásait vizsgáltuk. A gudron a pakura vákuumdesztillációját követően visszamaradó olajipari melléktermék. A Szlovák Köztársaságban a gudron lerakók régóta fennálló környezetterhelési kockázatbesorolást kaptak. A vizsgálatban a szennyezés forrásától négy különböző távolságból származó talajminták apoláris kioldható anyag (NES) tartalmát határoztuk meg, a talaj celluláz aktivitása és a talajlégzés mellett. A lerakók növényzetre gyakorolt hatásának vizsgálata a környező területek fitocönológiai felmérésével történt. A vizsgálatba bevont mindkét lerakó hosszútávú folyamatos szennyezést okozott, amit a NES monitorozás igazolt. A szennyezés terjedésének mértékét a NES koncentrációk változásával lehetett nyomonkövetni. Eredményeink alapján elmondható, hogy a két vizsgálati hely közül a Predajna 2 esetében a talajban jóval kiterjedtebb olajszennyezés volt megfigyelhető. A mintákban mért NES koncentrációk még a legtávolabbi minták esetében is jelentősen meghaladták a jogszabályban megengedett határértéket ($< 0,1 \text{ mg kg}^{-1}$). A talajlégzés vizsgálata során a termelődő szén-dioxid mennyisége összefüggést mutatott a NES által jelzett olajszennyezéssel. A magas koncentrációban lévő NES esetén az enzimatisz celluláz aktivitás gátlása volt megfigyelhető. A cellulóz enzimatisz lebontása csak alacsony NES koncentrációk esetén lehetséges, így csak a lerakótól legtávolabbi mintákban volt mérhető gyenge, illetve közepes aktivitás. Ezekben a mintákban mért mikrobiális aktivitás az olajszennyezés okozta gátló hatás kisebb mértékével indokolható. A talajban mért szennyezés mértékét a növényborítottsági adatok is visszaigazolták.

talaj celluláz aktivitás / talaj alaplégzése / nem poláros kivonható anyagok / olajfeldolgozásból származó maradékok / olajszennyezés

1 INTRODUCTION

Pollution by oil substances originating from anthropogenic activity has been an urgent and long-term global environmental problem. Large oil and oil product leakages into the components of the environment, especially water and soil (Wolińska et al. 2016), occur every year. Oil substances enter the environment in a variety of ways, e.g. leakages from oil wells, pipes, underground containers, and incorrect oil waste disposal (Kimes et al. 2014). Landfilling the residues from oil processing (gudrons) is an example of incorrect oil waste disposal. The waste – gudron (acid tars) – is produced during the refining of oil fractions with sulphuric acid. Gudron contains sulphuric acid as well as unwanted components removed from refined oil. In general, the composition of these residues depends on the composition of the oil (Speight 2006). In compliance with the valid legislation of the Slovak Republic (Regulation No. 365/2015 Coll.) gudrons are considered hazardous waste. They are dense, highly viscous compounds possessing an acrid, acidic smell. Gudrons are highly mobile and release sulphuric acid continuously (Tumanovsky 2004, Kolmakov 2006, Kreníková 2014). Gudrons are a persistent and unstable waste, typical for their toxicity, mutagenicity, teratogenicity, and carcinogenicity (Paluchova 2009, Masarovičová, 2013, Milne 2016). Gudron landfills are a threat to all parts of the environment. They pollute the air through the emissions they release during the summer months, pollute underground and surface water via leakages into surrounding areas, and also degrade the soil and contaminate the ore environment of the area in which they are present (Kreníková 2014). Soil health is not only important for people, but also for fauna and flora. Due to its sorptive and retentive properties, soil is a natural filter against pollutants circulating in the environment (Wyszkowski – Ziółkowska 2008). Contamination of the natural environment by oil substances contributes greatly to soil degradation. Though point sources contribute to contamination, it is the non-point sources of contamination that lead to the creation of integrated underground areas contaminated by these substances (Wyszkowski – Ziółkowska 2008). The environmental effect of oil substances on soil processes is the most visible in the activity changes of soil

microorganisms and enzymes (Li et al. 2007, Niemeyer et al. 2012), i.e. soil microbial activity can be considered a sensitive biological and biochemical indicator of soil quality (Margesin et al. 2000, Kaczyńska et al., 2015). Soil microorganisms are key elements in organic decomposition and mineralisation processes. Due to their quick reaction to changes and adaptation to the environmental conditions, soil microorganisms are often used as qualitative soil indicators. This is important for the preservation of favourable forest health. Soil microbiota is able to react quickly to stress in the environment (Nielsen – Winding 2002, Nannipieri et al. 2003, Fodor – Pájer 2017). Soil respiration (release of CO₂ by soil) and soil activity of cellulase belong to frequently monitored biological soil characteristics (Gömöryová – Fekiačová 2013). Šimek and Šantučková (2002) define soil respiration and cellulose decomposition as one of the basic microbiological characteristics that determine soil quality and health. Carbon dioxide, released by respiration from soil, is a final product of the microbial metabolism of organic remains (with the exception of decomposed organic matter). Basal soil respiration, measured as total release of CO₂, is an indicator of overall microbial activity in soil because the vast majority of prokaryotic and eukaryotic soil microorganisms obtain energy by oxidation from carbonaceous compounds. Basal soil respiration is also considered a rate of decomposition of mineral soil organic substances (Knoepp et al. 2000, Gömöryová et al 2013). Basal respiration is a main attribute related to fertility (Niemeyer et al. 2012) and a common indicator of soil quality (International Organisation for Standardisation 2002). Cellulose in the soil is commonly degraded by the cellulase enzyme, which is produced by microorganisms, usually by bacteria and fungi (Magnelli – Forchiassin, 1999). Cellulose is the most abundant organic compound in the biosphere, which contains nearly half of the biomass synthesised by the photosynthetic fixation of CO₂ (Eivazi – Tabatabai 1990, Eriksson et al. 1990, Tomme et al. 1995). Cellulose in soil originates mainly from the remains of plant matter, though fungi and bacteria in the soil also contribute limited amounts. The growth and survival of microorganisms depend on the source of carbon contained in the soil cellulose (Deng – Tabatabai 1994). To release carbon as an energy source, cellulose enzymes must degrade cellulose from plants to high-molecular oligosaccharides, cellobiose, and glucose. Within the process of systematic identification of environmental burdens, the following landfills were ranked among the environmental burdens of the Slovak Republic. Petrochema Dubová, a. s. as a refinery and petrochemical company processed oil using sulphonation and absorption technologies. Final products were made from obtained fractions, e.g. lubricating and special oils, detergents for laundry agents, and special white oils used in medicine and cosmetics. In the past, gudrons were placed in the natural environment and this led to the creation of two gudron landfills: Predajna 1 and Predajna 2 (Oravec 2014). This case study deals with the impact hazardous industrial waste – acid gudrons – from the Predajna 1 and Predajna 2 landfills have had and continue to have on soil and flora.

2 MATERIALS AND METHODS

This case study aims to assess the soil quality of areas contaminated by oil substances and evaluate soil basal respiration (CO₂), soil cellulase activity, analytical determination of non-polar extractable substances (NES), and vegetation conditions. The experiment took place in the monitored areas over two years (2018 – 2019). The monitoring was carried out in two research areas: Predajna 1 and Predajna 2 (the Slovak Republic, the region of Banská Bystrica), in two transects and four sampling points at distances of 0.5 m, 50 m, 70 m, 150 m from Predajna 1 (following the spread of pollution), and at distances of 1 m, 75 m, 110 m, 150 m from Predajna 2 (following the spread of pollution). GPS coordinates of the sampling points: Predajna 1 – 1. N48°49.197 E19°29.018, 2. N48°49.192 E19°29.016, 3. N48°49.186

E19°29.018, 4. N48°49.172 E19°29.029 and Predajna 2 – 1. N48°49.227 E19°28.702, 2. N48°49.195 E19°28.704, 3. N48°49.178 E19°28.715, 4. N48°49.151 E19°28.743 – Garmin GPS map 62sc. The distances for Predajna 1 and Predajna 2 are not identical due to the different configurations and accessibilities of the sampling points (*Figure 1*).

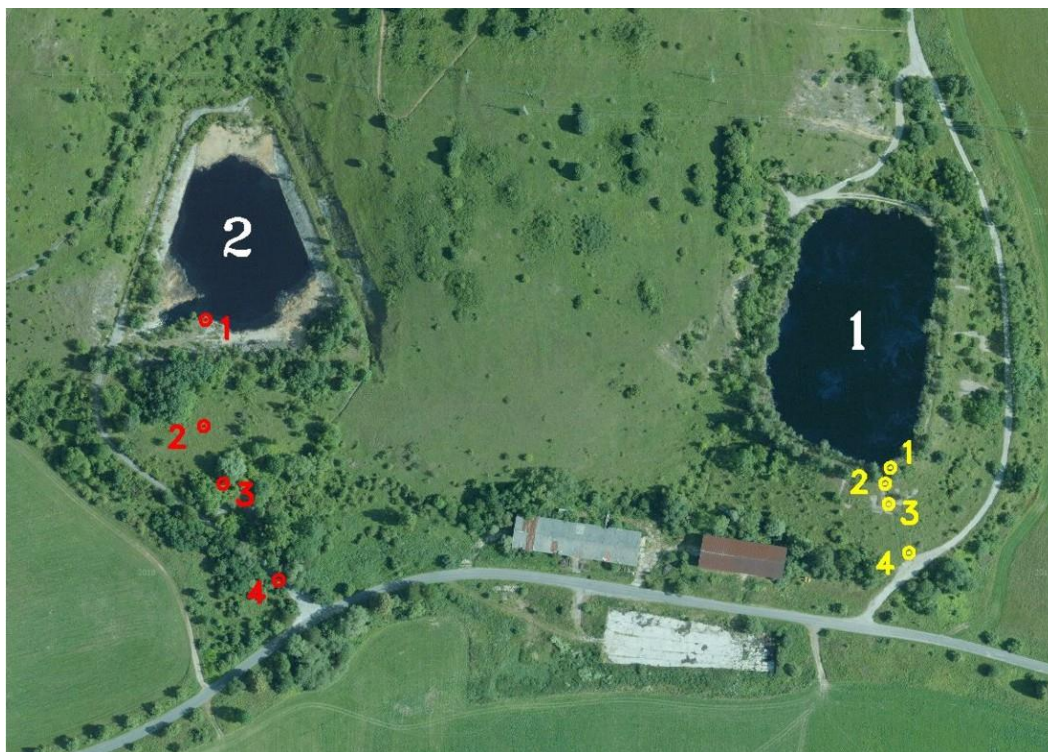


Figure 1. Map of the locality and the sampling points
 1 – Predajna 1; 1 - 0.5 m, 2 - 50 m, 3 - 70 m, 4 - 150 m
 2 – Predajna 2; 1 - 1 m, 2 - 75 m, 3 - 110 m, 4 - 150 m

2.1 Sampling

Five soil samples were taken five times a year during the vegetation periods in 2018 and 2019. A manual sounding stick was used to take the samples from the depth of 15-20 cm from each of mentioned areas (Regulation of the Ministry of Environment of the Slovak Republic No.1/2015 on uniform methods of analytical waste examination), without any effect of outdoor conditions (no rainfall total and average temperature of 20°C). Sampling was completed in compliance with STN ISO 10381-6, and the samples were treated by quartering to obtain a representative sample. In order to preserve natural soil character, the soil samples for basal soil respiration and soil cellulase activity measurements were not sifted.

2.2 Determination of basal soil respiration

Respiration or basal soil respiration by the Isermeyer method was determined by the amount of released CO₂ produced during an incubation period of 24 hours with the consequent titration with standardised volumetric solution of hydrochloric acid ($c = 0.05 \text{ mol L}^{-1}$), using phenolphthalein as an indicator. Data are expressed as $\mu\text{g CO}_2 \text{ g}^{-1}$ of dry soil (Alef 1991, Kizilkaya et al. 2004).

2.3 Determination of soil cellulase activity

The determination was completed according to Islam (1998), i.e. the principle is incubation of sterile cellulose in a Petri dish with a soil sample. After 30 days, quantitative decrease

was evaluated (in %) from the surface of cellulase using IMAGE J software (freely available on the Internet).

2.4 Determination of oil substances

Oil substances in soil were determined as non-polar extractable substances (NES) using spectrophotometer in an infrared area by the extraction with organic diluent (S-316) (Ladomerský 2001).

2.5 Vegetation research

Vegetation research was conducted in the form of phytocenological records in compliance with the Zürich-Montpellier School principles in the surroundings of gudron landfills in the vegetation periods of 2018 and 2019. Qualitative and quantitative characteristics were monitored in phytocenological relevés. Qualitative characteristics of plant community represent a set of all species that occurred in the studied area in the given period. The estimation of cover was assessed from quantitative characteristics, i.e. percentage estimation of the area covered by certain species. Places of phytocenological relevés are identical with the sampling points (Braun-Blanquet 1964, Moravec 1994).

2.6 Analysis of results

Using the program STATISTICA 12, ANOVA with interactions, confidence intervals of 95% and the program MatLab 2019b.

3. RESULTS AND DISCUSSION

This case study focuses on soil pollution in the surroundings of two gudron landfills, Predajna 1 and Predajna 2, which are environmental burdens in Slovakia. During exploratory works, 172,558 mg kg⁻¹ of NES was determined in gudron waste (Auxt 2018). Predajna 1 was built with a protecting dike in 1964 and was in operation until 1974. Predajna 2 was used for the deposition of gudrons from 1974 to 1983. The bedrock at the landfills consists of limestone, dolomites and rocks of melafire sequence. The bottoms of the landfills are not sealed, which led to a 60,000 ton gudron leak into the bedrock in 1982 (Ollerová 2004, Michaeli 2010).

3.1 Assessment of oil pollution

To assess soil samples in the surroundings of Predajna 1 and Predajna 2, the distances for sampling were measured from 0.5 to 150 m from the landfills. Monitoring of oil pollution spreading from the landfills revealed no significant differences between the NES values in the range from 0.5 to 150 m from the landfills in the years 2018 and 2019. Therefore, a stabilised situation can be assumed. Oil substance contamination decreases as distance from the landfill increases. Comparison of these two areas showed markedly higher oil substances pollution in the soil samples taken from the surroundings of Predajna 2 (*Table 3*). We presume that the determined high concentrations of NES are affected by the approximately 60,000 ton leak of gudrons into the bedrock in 1982 (Michaeli 2010). Precipitation amounts have also affected the long-term, gradual gudron contamination of the surrounding area from both landfills. Since both landfills are exposed, they are continuously replenished with rainfall, thereby increasing the environmental risk (Masarovičová 2013).

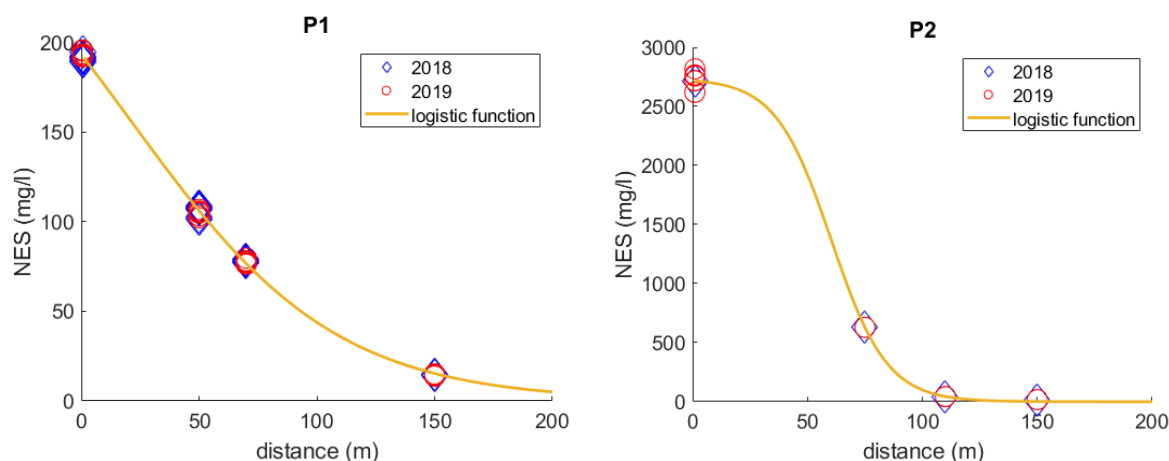


Figure 2. Estimation of the course of pollution depending on the distance
(Note: P1 – Predajna 1; P2 – Predajna 2)

In order to model data, the most suitable logistic function was:

$$y(x) = \frac{k_1}{1 + e^{k_2 x + k_3}} \quad (1)$$

Table 1 and Table 2 contain the results of non-linear regression calculated with the MatLab 2019b program. All coefficients in the Tables are statistically significant.

Non-linear regression model: $y \sim k_1/(1 + \exp(k_2 \cdot x + k_3))$ – Predajna 1

Table 1. Estimated coefficients-non-linear regression parameters Predajna 1

	Estimate	SE	tStat	pValue
K1	2736	8.8003	310.9	$2.5 \cdot 10^{-99}$
K2	0.0827	0.0040	-20.34	$7.0 \cdot 10^{-29}$
K3	-4.99	0.3090	16.15	$1.0 \cdot 10^{-23}$

Number of observations: 64, Error degrees of freedom: 61, Root Mean Squared Error: 24.8
R-Squared: 1, Adjusted R-Squared 1; F-statistic vs. zero model: $6.75 \cdot 10^4$, p-value = $2.58 \cdot 10^{-107}$

Non-linear regression model: $y \sim k_1/(1 + \exp(k_2 \cdot x + k_3))$ – Predajna 2

Table 2. Estimated coefficients – non-linear regression parameters Predajna 2

	Estimate	SE	tStat	pValue
K1	312.16	6.6699	46.8	$1.6 \cdot 10^{-49}$
K2	0.02297	0.0004	-59.5	$9.9 \cdot 10^{-56}$
K3	-0.49	0.0543	8.98	$9.2 \cdot 10^{-13}$

Number of observations: 64, Error degrees of freedom: 61, Root Mean Squared Error: 1.75
R-Squared: 0.999, Adjusted R-Squared 0.999; F-statistic vs. zero model: $9.49 \cdot 10^4$, p-value = $7.69 \cdot 10^{-112}$

When assessing the results, we focused on the evaluation of the course of pollution according to distance. The logistic function predicts the course of pollution (based on the NES indicator) to increasing distance from the pollution source (Predajna 1 and Predajna 2).

Table 1 - 2 and Figure 2 show that the NES content in the soil samples taken at a distance of 1 m from Predajna 2 was 14 times higher than it was in the soil 0.5 m from Predajna 1. Only the 150m distance from both landfills showed no significant difference between the determined values of NES for both areas. Higher content of NES was detected in analysed

soil from the surroundings of Predajna 2 at the distances of 1 m and 75 m. However, the spread of oil pollution at the distances of 110 m and 150 m decreased to nearly comparable levels of pollution in the sampling points of P1 (Note: P1- Predajna 1; P2 – Predajna 2). Determined content of NES did not meet the criteria of permissible concentration in soil samples, not even at the 150 m distance ($< 0.1 \text{ mg kg}^{-1}$ in compliance with the Law No. 220/2004 Coll.).

Table 3. Characteristics of non-polar extractable substances (mg kg^{-1})

	Predajna 1			Predajna 2		
	Distance (m)	Average	St. Error	Distance (m)	Average	St. Error
2018	0.5	191.63	0.20	1	2715.31	0.30
2018	50	105.68	1.41	75	629.53	0.28
2018	70	78.07	0.18	110	43.12	0.07
2018	150	14.75	0.07	150	17.57	0.19
2019	0.5	193.77	0.74	1	2716.69	0.50
2019	50	105.00	0.54	75	629.47	0.14
2019	70	77.94	0.20	110	43.36	0.40
2019	150	14.66	0.15	150	17.97	0.13

3.2 Assessment of basal soil respiration

Oil pollution degrades physical and chemical soil characteristics. Greasy film on the soil surface limits air circulation between the soil and the atmosphere. Soil particles coated by oil hinder CO_2 from leaving the soil for the air. Oil pollution also degrades the biological properties of soil (Frankovská 2010, Samešová 2011). Changes in soil caused by oil pollution were also monitored on the basis of determination of basal soil respiration. In the analysed samples (Figure 3, Table 4) the content of determined CO_2 corresponds with determined NES values (Table 3): as presented by Hybská et al. (2013), basal soil respiration grows with decreasing NES content. In their scientific study, Polyak et al. (2018) dealt with the monitoring of basal soil respiration in soil burdened by oil pollution. They confirmed that CO_2 production corresponds with the course of degradation of oil pollution controlled by the determination of petroleum hydrocarbons. Ali et al. (2020) found that the rate of CO_2 development in the soil burdened with oil pollution ranged from 30.6 to $55.0 \text{ } \mu\text{g CO}_2 \text{ g}^{-1} \text{ day}^{-1}$.

Table 4. Statistical characteristics of basal soil respiration ($\mu\text{g CO}_2 \text{ g}^{-1} \text{ day}^{-1}$) results in the monitored locality

Year	Predajna 1					Predajna 2				
	Distance (m)	Average	St. Error	Confidence intervals		Distance (m)	Average	St. Error	Confidence intervals	
				-95%	95%				-95%	95%
2018	0.5	11.98	0.09	11.70	12.25	1	1.16	0.01	1.11	1.21
2018	50	16.06	0.05	15.88	16.23	75	7.11	0.02	7.06	7.17
2018	70	23.08	0.20	22.43	23.72	110	35.69	0.12	35.31	36.06
2018	150	43.43	0.18	42.86	44.00	150	40.24	0.18	39.65	40.83
2019	0.5	12.23	0.14	11.76	12.69	1	1.22	0.04	1.11	1.34
2019	50	16.15	0.13	15.73	16.56	75	7.11	0.01	7.07	7.15
2019	70	23.91	0.52	22.25	25.56	110	35.71	0.08	35.47	35.95
2019	150	43.25	0.24	42.48	44.01	150	40.56	0.14	40.12	40.99

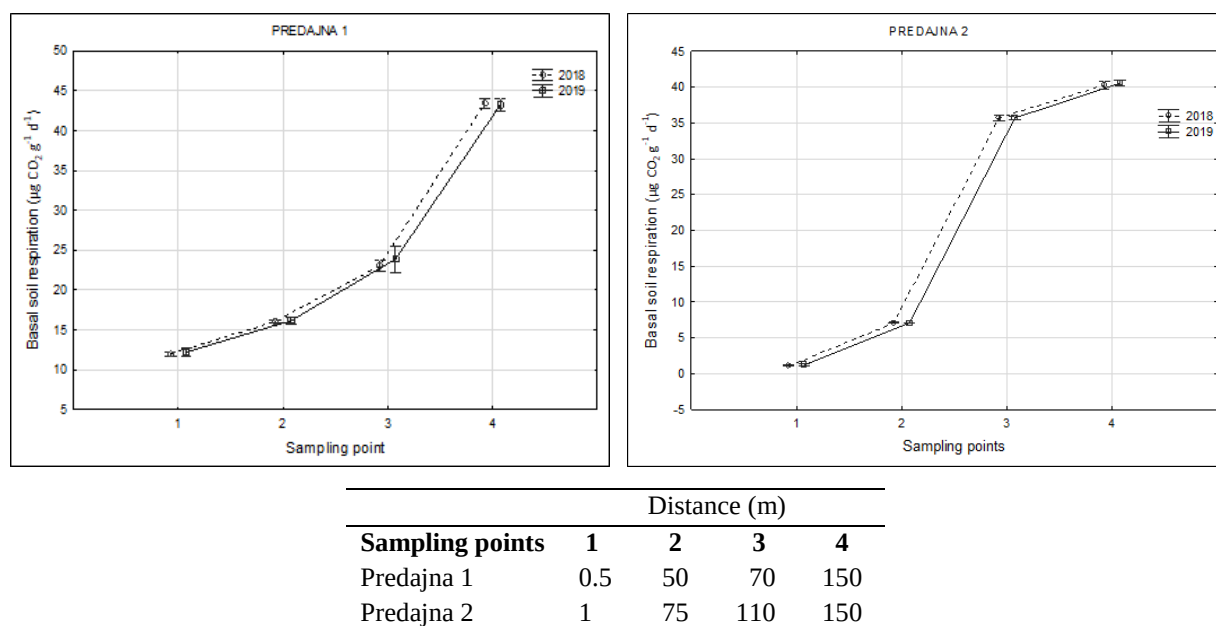


Figure 3. Graphical representation of basal soil respiration results

3.3 Assessment of soil cellulase activity

Polysaccharide cellulose is a basic component of plant tissue cell walls and is also the most common organic compound in the biosphere. After plants die, cellulose is decomposed by the enzymes that belong to the group of cellulases. Anthropogenic effects of oil pollution were assessed by the determination of the rate of soil cellulase activity. Cellulose was not decomposed in the most polluted soil samples (*Table 5*). Enzymatic cellulase activity was detected only at the lowest NES concentrations. According to Rejšek (1999), there is no cellulolytic enzyme activity in soils with the highest NES concentrations. It is possible to range the soils of lower NES concentrations (soils taken from the distances of 70 m and 150 m from Predajna 1; 110 m and 150 m from Predajna 2) among the soils with weak or middle activity of soil cellulase (*Figure 4*, *Table 5*). This means that microbial activity was detected in the soil samples and its values were higher due to the oil pollution-caused decrease of inhibitors. The results of this case study correspond with Hybská et al. (2013), where the percentage rate of the soil cellulase activity in the soil contaminated by oil was 2.12%. In soil contaminated by synthetic oil (completely or very well degradable oil), this figure was 8.72% (Fargašova 2009) while it was 15.95% in soil without oil pollution. For the comparison, the results of Javorekova et al. (2006) can be mentioned: the cellulose decomposition was detected at the depth of 0.1 m in agricultural soil (44% in brown earth and 35.15% in black earth). Compared with our results, obtained under the same conditions as mentioned in Javorekova (2006), high NES concentrations inhibit the enzymatic activity in such burdened soil (*Table 3*).

Table 5. Statistical characteristics of cellulase activity results in the monitored locality

Year	Predajna 1					Predajna 2				
	Distance	Average	St.	Confidence intervals		Distance	Average	St.	Confidence intervals	
	(m)	(%)	Error	-95%	95%	(m)	(%)	Error	-95%	95%
2018	0.5	0.00				1	0.00			
2018	50	0.02	0.01	0.00	0.05	75	0.00			
2018	70	0.35	0.02	0.30	0.39	110	2.92	0.03	2.81	3.03
2018	150	0.90	0.00	0.89	0.91	150	1.83	0.45	0.39	3.26
2019	0.5	0.00				1	0.00			
2019	50	0.02	0.00	0.01	0.03	75	0.00			
2019	70	0.36	0.01	0.31	0.40	110	2.45	0.49	0.91	4.00
2019	150	1.04	0.01	1.01	1.06	150	1.45	0.02	1.40	1.50

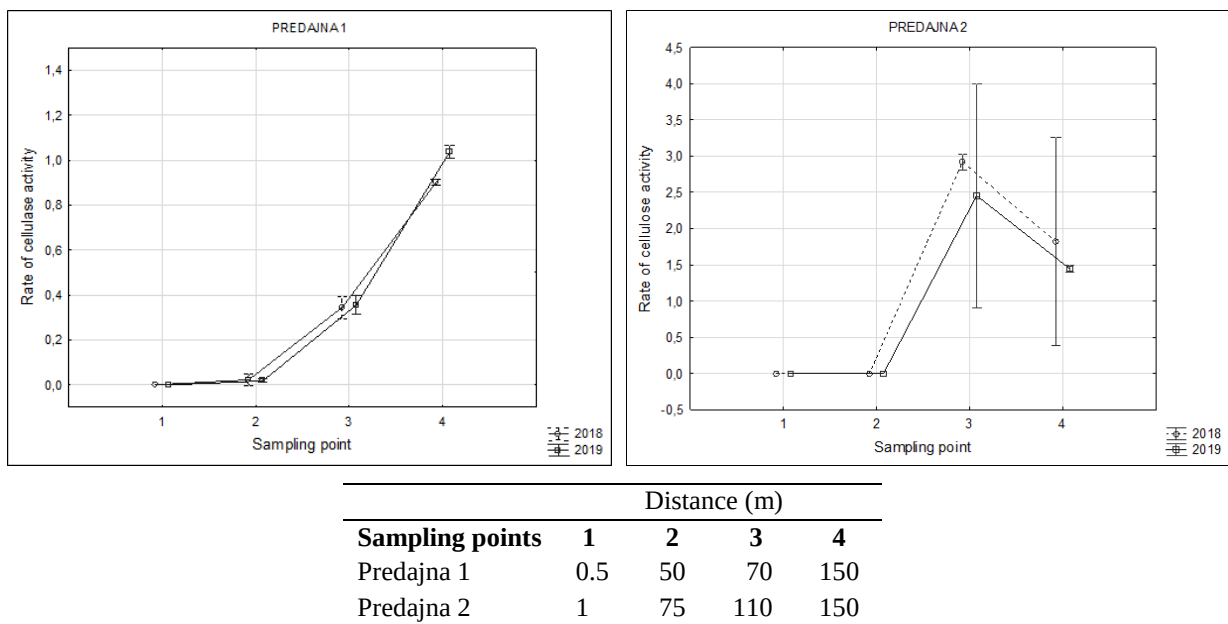


Figure 4. Graphical representation of cellulase activity results

3.4 Assessment of vegetation conditions

Several factors affect basal soil respiration and soil cellulase activity. Based on phytocenological records from 2018 and 2019, it is possible to state that the surface cover percentage increases with the increasing distance from the landfill border. Surface vegetation cover increased from 30% to 90% at Predajna 1 in 2018 and from 40% to 100% in 2019. At Predajna 2, the cover increased from 50% to 100% in both years of monitoring (Table 6). The number of species at Predajna 1 stayed identical or increased slightly with distance. In 2019, the number of species increased to 20 in the area closest to the landfill (in comparison with 8 in 2018). However, the cover only increased by 10%. No vegetation was detected in the area without any determined cover. A trench from the landfill border over the dike to the root of the dike was created there. It is possible that gudron waste was flowing away in that direction when landfill levels were high. This can be related to water erosion and excessive precipitation. A doubling of the amount of species does not automatically indicate a larger creation of biomass and higher cover. At Predajna 2, the higher number of species due to distance was not detected. However, total cover grew from 50% to 100%. Cellulase activity increased with distance from the landfill, as well as total vegetation cover of the area. Higher

cover leads to the higher the production (creation) of surface and underground biomass. Consequently, the amount of waste and dead and decayed biomass rises as well.

The occurrence of the woody plants *Betula pendula*, *Salix caprea*, and *Populus tremula* was detected near the landfills. Herbs *Calamagrostis epigejos* and *Dactylis glomerata* achieve cover ranging from 25% to 50%. *Arrhenatherum elatius* and *Rubus caesius* cover is from 50% to 75%. With the exception of *Arrhenatherum elatius*, Pyšek (1981) and Hartman (1980) consider these herbs to be oil pollution tolerant. The occurrence of *Calamagrostis epigejos* was observed in the areas closest to the landfills where the highest NES content was also detected. According to the above-mentioned authors, *Arrhenatherum elatius* belongs to a group of species that are sensitive to oil pollution. This species did not occur in the areas where the highest NES concentrations were detected.

Table 6. Characteristics of vegetation in the monitored locality

Year	Predajna 1			Predajna 2		
	Distance (m)	Number of species	Total cover (%)	Distance (m)	Number of species	Total cover (%)
2018	0.5	8	30	1	12	50
2018	50	10	70	75	19	90
2018	70	0	0	110	20	80
2018	150	9	90	150	14	100
2019	0.5	20	40	1	11	50
2019	50	13	80	75	22	100
2019	70	0	0	110	19	70
2019	150	13	100	150	13	100

4. CONCLUSIONS

Based on the obtained results of this case study, we can conclude that gudron waste is an environmental burden for soil. This was proved by the presence of non-polar extractable substances (NES) in soil samples at different distances from the landfills. Contamination decreased in accordance with increasing distance. The assessment of the course of oil pollution based on the NES indicator according to distance is predicted by a logistic function. Cellulose was not decomposed in soil that was closest to gudron landfills. Soil activity increased as distance from the landfills increased. Higher microbial activity was detected in the samples where inhibitors were lowered due to the pollution. Basal respiration highlights the ability of microorganisms to use available substrate, especially organic matter. Basal respiration determination is significant in soils affected by different negative factors, e.g. negative anthropization (in this case oil pollution). CO₂ production rose as distance from the landfills increased. This corresponds with the course of degradation of oil pollution controlled by the determination of non-polar extractable substances. Based on the phytocenological records from 2018 and 2019, we conclude that percentage cover of the area increases as distance from the landfill border increases. Determination of basal soil respiration and cellulase activity were confirmed as suitable indicators for the monitoring of oil polluted soil. Based on these findings, we recommend the assessment of basal soil respiration as a suitable monitoring method to measure soil contamination by oil substances. This method is more financially and environmentally accessible than NES monitoring. Monitoring of these indicators is also important in forest ecosystems.

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The Standard Output of Forest Index – an Indicator of Site Quality

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Abstract – The Standard Output of Forest Index (SOFI) describes the ability of forests to produce financial value from wood production based on the standardized monetary value of the mean annual increment of the potential final harvest relative to a reference forest type. It can be applied on regions where the forests can be classified into major tree species or species groups and into site classes. The potential volume of final wood harvest is estimated through yield tables. Using the share of low-quality and high-quality wood product groups in the final harvest, and their respective standardized price, the output value of the final harvest is expressed and then divided by the rotation age. This standardized output is compared to a reference forest type identified by its tree species and site class, and multiplied by 10 points. The SOFI of the reference forest, therefore, is 10, while higher values represent higher potential output and smaller values represent smaller potential output. With the necessary modifications, the SOFI can be applied to uneven-age forests as well. It can primarily be used to describe and compare the financial output potential of larger forest areas.

Site index / site classes / forest productivity / wood quality / SOFI / forest economics

Kivonat – Erdészeti termelési érték index – a termőhelyi potenciál mutatója. Az erdészeti termelési érték index (SOFI) az erdők fatermesztésből származó pénzérték termelő képességét jellemzi a korszaki átlagnövedék standardizált pénzügyi értékének kifejezésével és egy meghatározott referencia erdőtípushoz történő viszonyításával. Olyan földrajzi régiókban alkalmazhatók, ahol az erdők főbb fafajok, illetve fafajcsoportok és fatermési osztályok szerinti csoportokba sorolhatók. A potenciális véghasználati fahozam fatermési táblák alapján becsülhető. Az alacsony és magas minőségű fatermékek véghasználati hozamon belüli arányára és azok standardizált pénzértékére alapozva a véghasználati kibocsátási érték kifejezhető, amelyet a vágáskorral osztunk. Ez a standardizált termelési érték viszonyítandó egy fafaj és fatermési osztály által meghatározott referencia erdő értékéhez, és a könnyeb megjeleníthetőség érdekében 10 ponttal szorozzuk. Ezáltal a referencia-erdő SOFI értéke 10, míg a magasabb értékek magasabb potenciális termelési értéket, az alacsonyabb értékek alacsonyabb potenciális termelési értéket jelentenek. Megfelelő módosításokkal a SOFI többkorú erdőkre is alkalmazható. Elsősorban nagyterületű erdők pénzügyi kibocsátási potenciáljának leírására és összehasonlítására használható. Elsősorban nagyobb erdőterületek potenciális kibocsátásának jellemzésére alkalmazható.

termőhelyi index / fatermési osztály / termőhely jósága / termőképesség / fa minősége / SOFI / erdészeti ökonómia

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1 INTRODUCTION

This paper introduces the Standard Output of Forest Index (SOFI) by presenting its definition, calculation method, and the rationale of application. The aim of the introduction of the SOFI is to provide an index which can represent the extent to which a forest site is suitable for wood production. The index measures both the quantity and quality of a site relative to other sites by providing a financial basis comparison over various tree species and forest types.

The ability of a forest site to produce wood is generally understood as its site quality. Better site quality results in higher volume of wood in a given period of time, which can be expressed through standing volume, total wood production, or other features. Site index is commonly used to describe site quality, which is the height of the dominant trees at a reference age. The assumption that height growth strongly correlates with the increment of the growing stock has been used since as early as the end of 19th century (Baur 1881) to describe forest growth and to predict future wood production potential.

If the height-to-age relationship of a tree species or forest type is known, the site index can be calculated at any age on the growth curve's domain. The site index can be grouped into site classes in order to divide the height-to-age continuum into distinct categories, simplifying the description of site quality considerably. Site classes can be characterized by their growing stock, stem number, basal area, annual increment, and mean diameter as the function of age of the tree stand. Such datasets can be found in yield tables. Although the fundamental assumptions of such yield tables and their application on large regions has been challenged and proved to be inadequate under certain management regimes (Skovsgaard – Vanclay 2008), they are widely used in practice.

Site quality is the interplay of forest site properties and tree stand type. The forest site can be described by its climate (the combination of temperature, precipitation, and other factors), hydrological properties, soil (type, depth, texture, and nutrition content), while the most important tree stand characteristics are tree species composition (provenance may also be considered) and establishment method (seed or coppice, spacing). Therefore, the site index can only be used for comparison within tree species or tree species groups.

This study does not directly address possible site condition shifts. Should these occur in over the long term, their effects on future growth potential need to be considered. Detectable increase in the forest growth rate was reported in some areas (Somogyi 2008, Pretzsch 2014), while forest cover extinction is projected at the xeric limits of forests in other areas (Mátyás et al. 2014). Climate change is one of the large-scale causes of such changes, which can be coupled with changes in hydrological conditions (Csáki et al. 2014, Moricz et al. 2016).

The advantage of the site index is that tree stand height is less influenced by forest management interventions, e.g. thinning, than by other stand characteristics, e.g. number of stems, diameter etc. However, the application of the site index is confined to situations when forests are even-aged and their age can be determined by samples or from planting records. These latter obstacles can easily be tackled in areas with long traditions of forestry and forest cultivation, as forest inventories in such areas tend to possess over 90% coverage (MacDicken et al. 2015). Other areas require alternative methods based on features other than age.

In forests of differing tree species, comparisons of wood productivity can be based upon mean annual increment (MAI) at a reference age representing the typical rotation age of the respective tree species in the described region. Increment is normally measured in volume (cubic meters), but a more accurate comparison can be achieved if the increment is expressed in dry matter weight (tons). Since the dry weight of wood strongly correlates with its carbon content (Lamolm – Savidge 2003), the dry weight of MAI is not only an indicator of wood production capacity, one that allows for cross-species comparison, but can also express the aboveground carbon sequestration capacity of forests. (Gallaun et al. 2010)

The above approaches to site quality imply that larger natural output in volume or dry matter under the same period of time contributes to higher financial output. To describe and compare the financial potential of forests as an alternative understanding of site quality entails considering the market value of wood products. Based on growth information and the forest management regime, the financial flows for a given time period can be modelled and used to calculate economic properties such as income, added value, margin or profit, etc. However, these calculations are either applied in regions of homogenous forest types under similar forest management regimes and are based on necessary simplifying assumptions (Pandey et al. 2010), or they need to gather exhaustive amounts of data and build large numbers of models. The data collection method determines the comparability of economic features and limits large scale analysis (Sekot et al. 2011, Vrolijk et al. 2016).

Site quality is a feature of a specific site, and its indicators express its production capability, which is an important component of a site's land value. However, this feature should not be treated as a single component, as it is, to a great extent, influenced by the utilization forms, forest management regimes, production risks, organizational arrangements, management objectives, etc. (Hartebrodt 2007, Posavec 2017, Beljan et al. 2018).

2 THE STANDARD OUTPUT OF FORESTRY INDEX

2.1 Aim, principles and definition

The aim of the SOFI is to provide a site quality index that considers the potential quantity and quality of wood production and allows for comparisons both within and across tree species.

The principles of the index design:

- As long as the representative power of the index is not seriously corrupted, a simple calculation method takes precedence over accuracy.
- Meant to be an indicator of the potential financial performance of forests, rather than an exact economic variable.
- Describes the variability of forest type and forest site combinations rather than the differences in the financial value of wood in different regions.

Definition: the Standard Output of Forest Index describes the ability of forests to produce financial value through wood production based on the standardized monetary value of the mean annual increment of the final harvest relative to a reference forest type.

2.2 Calculation method and data source

Step 1: Forming forest categories

Within a defined geographical area where the SOFI will be employed, forests shall be categorized according to major tree species, species groups, or sub-species categories (e.g. selections or provenances) for which growth and wood price data are available. Groups shall be formed with due consideration of their share in forest cover and the availability of the data specified in the next steps.

Step 2: Determining final harvest volume and rotation age

There is no restriction on how to determine potential final harvest volume and final harvest age, but yield tables based on site classes are the most commonly available tools. If available, silviculture models can also be a good data source. Theoretically, rotation age can be determined at the forest plot level, but this data is unlikely to be available in young forest stands. Therefore, it is best to unify rotation age on the whole geographical area of application

(or at least at the regional level) by tree species and site index classes, which represent the best financial interest of the forest owner/manager under the relevant legal framework.

Step 3: Determining the share of high-quality and low-quality wood product groups

The calculation of the SOFI requires the proportion of low-quality wood products be known. There is no clear distinction between low quality and high-quality wood products. Firewood and pulp and paper wood typify the former, while saw logs and veneer logs belong to the latter. In general, there are usually no quality requirements for low-quality products other than they consist of healthy solid wood, and that they are measured in stockpiles. High-quality products are inspected against quality requirements and measured piece by piece. If in doubt as to where a specific wood product belongs, price is decisive. The definition of wood products and their grouping may follow national or international standards, but it shall correspond to the wood product classification applied in wood price data sources described in Step 4. The share of the wood product groups shall represent averages of a 5-10-year period.

Step 4: Selecting representative wood products for the high- and low-quality groups and standardizing their monetary value

For each of the high and low-quality product groups, a representative product that has the highest total output value within the respective product group shall be selected. The value of wood products shall be expressed in monetary terms, usually those stated in controlled markets such as commodity exchanges, farm accountancy data networks, or national statistics; however, in the absence of these, individual surveys can become possible sources of price information. The data source, however, is suitable only if prices of the most important wood products can be obtained for a time period no shorter than five years. In the calculation of the SOFI, the 5–10 year average of these prices shall be used.

Step 5: Calculating and indexing to a selected forest type

The standard output for each forest category shall be calculated according to eq. 1. Final harvest volume is multiplied by the weighted average value of wood and divided by the rotation age. As an alternative, eq. 2 can be applied in cases when rotation age and the final harvest volume are unavailable, but the mean annual increment can be estimated.

One of the forest categories shall be designated to serve as the basis of indexing. This reference forest shall cover a relatively large area and will be selected from around the middle of the standard output spectrum. For a simpler presentation, the proportion of the standard output of the specific forest category to the reference category is multiplied by 10 points. Thus, the unit of SOFI is ‘points’.

$$\text{SOF}_x = \frac{[\text{SV}_{LQx} \cdot \text{LQ}\%_x + \text{SV}_{HQx} (1 - \text{LQ}\%_x)] Q_x}{R_x} \quad (1)$$

$$\text{SOF}_x = \overline{\text{SV}}_x \cdot \text{MAI}_{Rx} \quad (2)$$

$$\text{SOFI}_x = \frac{\text{SOF}_x}{\text{SOF}_{ref}} \cdot 10 \quad (3)$$

Where:

- x : mark of forest categories (combinations of species groups and site classes)
- ref : mark of the reference forest category
- SOF : Standard Output of Forest
- SOFI : Standard Output of Forest Index
- SV_{LQ} ; SV_{HQ} : standard value of the representative wood product in the low-quality and high-quality forest product groups
- $\text{LQ}\%$: share of low-quality forest product group in the final harvest
- Q : potential standing volume of wood at final harvest

R:	rotation age
MAI _R :	mean annual increment of the potential final harvest
$\overline{SV}$:	weighted average value of the low- and high-quality forest product groups

Step 6: Application to the forest area

The SOFI can be applied to forest areas where species composition and site classes (or other parameters that have been used for classification at Step 1) are known. The minimum level of application corresponds to that of the natural parameters, which is, most commonly, the forest sub-compartment; however, this can be aggregated to larger areas as well.

3 CONSIDERATION OF DESIGN ALTERNATIVES

The actual financial performance of a forest depends on the harvested volume, the market value of its products, and the production costs, which includes harvesting, (re-)establishment, planning, supervision, and other administrative tasks. Surveying relevant data becomes a demanding task if the actual financial performance is to be presented or used in an analysis. This is especially pertinent in larger geographical areas with varied forest stand types and forestry practices.

This is also valid if the potential economic performance needs to be taken into account. In this case, potential harvest volume can be interpreted as the allowable cut within the time horizon of the study based on the current state of the forests depending on their age, volume, stocking, health status, management objectives, conservation, and other limitations. Another option is to make estimations on the future potential based on the combinations of the site conditions and tree stand type. Going even further, if the current tree stand is, for some reason, unable to utilize the full production potential of the site due to factors like bad species in the past, then an alternative forest type with highest potential production can also be used for the calculation.

The SOFI is meant to represent the suitability of the forest area to produce financial value based on the current site and forest type combinations. It aims at considerably simplifying data collection, modelling, and calculation tasks by reducing the necessary amount of data, while providing a good approximation of the potential financial performance of a study area. This is why the most schematic method was incorporated into the calculation method from among the options described above.

Final harvest volume represents a forest's wood production capacity. Excluding the premature wood production renders the SOFI biased because the share of premature wood in total wood production varies significantly in the different tree species groups, as observed in *Table 1*.

Simply replacing final harvest volume with total wood production would also raise concerns regarding financial valuation as thinnings produce wood products of lower quality and smaller dimensions compared to final harvest products. Extending the calculations by incorporating the estimation of the monetary value of the premature harvests would require further estimations of the share of wood product groups. The dilemma of whether the approximation of the output should be based on a more detailed calculation, or the calculation should be kept the simplest possible, has been decided in two arguments. First, one of the principles of the SOFI is to avoid detailed modelling and obtain only the smallest number of absolutely necessary factors. Second, timber harvests are generally not subject to market circumstances only, but to non-economic considerations as well (Kilham et al. 2019). This is even more applicable to premature harvests because their expected profit is smaller than that of the final harvest; therefore, their contribution to the 'output potential' is questionable.

Table 1. The share of the final harvest volume in the total wood production of selected species based on their yield tables (Source of data: Béky 1981, Keserű and Rédei 2012, Rédei et al. 2019)

Tree species	Wood production category	Site classes					
		I.	II.	III.	IV.	V.	VI.
Oak	Total wood production at age 100 (m ³ /ha)	1056	905	762	630	509	394
	Final harvest volume at age 100 (m ³ /ha)	595	510	432	357	288	222
	Proportion of final harvest in the total production (%)	56.3	56.4	56.7	56.7	56.6	56.3
Black locust	Total wood production at age 30 (m ³ /ha)	463	387	316	252	195	173
	Final harvest volume at age 30 (m ³ /ha)	304	252	204	162	124	107
	Proportion of final harvest in the total production (%)	65.7	65.1	64.6	64.3	63.6	61.8
Hybrid poplar	Total wood production at age 20 (m ³ /ha)	412	349	298	247	201	163
	Final harvest volume at age 20 (m ³ /ha)	341	276	228	187	151	121
	Proportion of final harvest in the total production (%)	82.8	79.1	76.5	75.7	75.1	74.2

It is, therefore, left to the user to decide whether to use the original form of the SOFI or to modify it according to the available data. One alternative modification is presented in *eq.4*, which would adequately reflect the forestry practice in hardwood regions, but would not necessarily be suitable elsewhere. *Eq.4* is a modification of *eq.1* by adding the premature harvest potential at the standard value of low-quality wood products, as if 100% of the premature wood harvest belonged to the low-quality product group.

$$SOF_x = \frac{[SV_{LQx} \cdot LQ\%_x + SV_{HQx} (1 - LQ\%_x)] Q_{FHx} + SV_{LQx} \cdot Q_{PHx}}{R_x} \quad (4)$$

Where symbols are the same as at *eq.1*, except:

Q_{FHx} : Volume of final harvest

Q_{PHx} : Volume of premature harvest

While natural aspects such as final harvest volume represent the specific site, the economic layer (distribution of wood products and prices) is standardized. This is justified by the circumstance that the primary aim of SOFI is to represent forest capability. Thus, the effects of the regional differences in the economy (i.e. the wood industry and other related sectors) and the customs of direct household consumption should be excluded. This is the reason for calculating with mid-term or long-term average wood product distribution and prices. These factors are unified for the whole study area.

Although forests can produce a large variety of products and services, wood products remain one of the most important sources of income (Sisak et al. 2016). Among various factors, the actual wood product distribution of a specific final harvest depends on tree quality, tree dimensions, market demand, and forest manager preferences (e.g. own consumption needs). To tackle this problem, wood products are grouped into low-quality and high-quality groups, thereby eliminating the effects of minor changes. Calculating with the average product distribution over a 5-10-year period levels short-term volatility.

One might notice that the shares of low-quality and high-quality wood products are applied to the gross final wood harvest even though their shares refer to the net wood harvest. This can be seen as an inconsistency; however, the SOFI is meant to be an indicator. The

conversion of gross wood harvest to net wood harvest would be necessary only if the conversion rate of the forest types and site classes were significantly different. This type of modification is possible depending on the purpose and area of the application.

Selecting the market price source of the representative wood products requires careful consideration. It is of paramount importance to avoid the effects of short-term price volatility that would distort the comparability of forest types. Wood products tend to show constant prices relative to each other (Rumpf et al. 2015); however, this is only true on longer time horizons. There are well established economic survey networks aiming at surveying incomes and costs at forest holdings (Schiberna et al. 2011, Sekot 2017). In these cases, prices need to be calculated to the same delivery point (e.g. in the forest or delivered to factory etc.) before they are used to calculate the mean values.

The calculations exclude production costs. Through the incorporation of production costs, a profitability index could be created, one which would give a more precise indicator of the financial potential of forests. Nevertheless, costs are much more uncertain than incomes. Some of the costs depend on natural circumstances, such as the terrain as well as type of forest and site. Other costs are influenced by local economic conditions such as transport infrastructure, market accessibility, the labour market, and other resources as well as forest manager choices and legal restrictions. These factors make cost estimations uncertain enough to divert the index from its original purpose of remaining simple and of representing forest and site combinations rather than regional economies.

It is worth repeating that the SOFI can be further adapted to the purpose of its application and to data availability, and extended with the major cost items similarly to *eq.5*, which considers reforestation as a crucial cost.

$$\text{SOF}_x = \frac{[SV_{LQx} \cdot LQ\%_x + SV_{HQx}(1 - LQ\%_x)] Q_x \cdot (1 - C\%)}{R_x} \quad (5)$$

Where symbols are the same as at *eq.1*, except:

C%: Share of reforestation costs to output value of final harvest

Output potential approximations become more accurate with larger aggregation areas and forests that fit the definition of a normal forest. The current state of a forest does not influence the result as its tree species composition is the basis of calculation, but its stocking, health, and other properties are not considered.

The greatest advantage of the SOFI is the ability to compare various tree species, and the effect of the quality premium of wood products. Although the calculation is primarily based on yield tables, which are constructed for even-aged forests, *eq.2* shows that if MAI is available for uneven-aged forests, these can also be covered. In this case, MAI should refer to mature trees only, in the same manner that only the final harvest is considered for even-aged forests.

Similar to the case study below, indexing the SOFI is unnecessary if it is used in calculations. The idea behind choosing a reference forest type is that in long time series the nominal values of the SOFI need further processing to be comparable (because of price inflation for instance). Furthermore, when the SOFI is used to present the suitability of forest for wood production, it is easier to demonstrate in comparison to a frequently occurring forest type than with an indicator with an abstract meaning.

4 DEMONSTRATION OF APPLICATION

In an attempt to demonstrate the applicability of the SOFI and its ability to represent the financial potential of a forest, a simple case study is presented here. The hypothesis of this study is that the income from the forest is strongly correlated with the potential output, which can be described with the SOFI replacing detailed modelling.

There is no common time period for which all data necessary for the calculations would be available; therefore the following sources were used:

- National Forest Database from 2012, which contains tree species distribution and site classes at the forest plot level, and the geographical location of the plot (NFD 2012)
- Yield tables for the forest species groups (Béky 1981, Béky 1983, Kovács 1983, Kiss et al. 1985, Solymos 1993, Keserű – Rédei 2012, Keserű et al. 2017, Rédei et al. 2019)
- National Statistical Program annual survey on forest products 2010–2015
- National Statistical Program annual survey on wood product prices 2018
- Wood product price-surveys of the NARIC Forest Research Institute 2013–2018.

In Hungary, there are 22 state-owned forest companies covering the whole country. Excluding those which have woodworking branches, or which do not cover an entire county, 12 can be used as samples and be compared to the SOFI value of the respective county.

The SOFI is calculated for tree species groups divided into six site classes. The reference forest type is Turkey oak (*Quercus cerris*) third site class, which provides a relatively high MAI, but does not comply with higher quality standards due to the low level of wood resistivity.

Forest companies disclose their incomes publicly in their annual financial reports. For this demonstration, their mean annual income per hectare from 2010–2014 is used. *Figure 1* shows that the company income per hectare is strongly correlated ($R^2=0.85$) with the SOFI of the respective county.

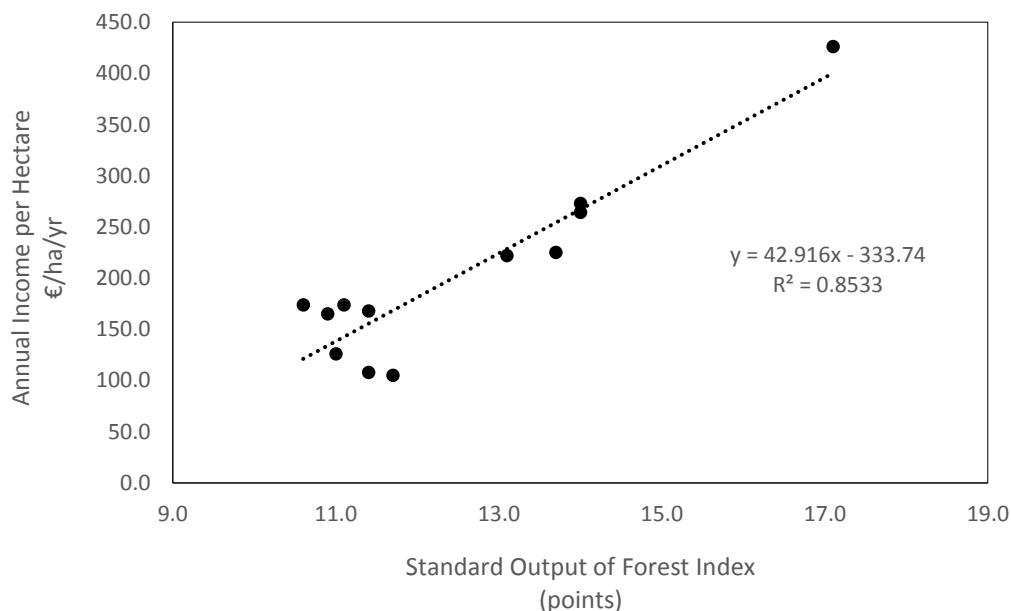


Figure 1. Relationship of the annual income per hectare (€/ha/yr) of selected forest companies and the Standard Output of Forest Index of the counties where their forests are located (Currency rate: 333 HUF/EUR)

5 CONCLUSIONS

If the potential financial performance of a defined forest area is to be described, the SOFI can be used efficiently by reducing the necessary input data and modelling. The need for such reduction arises from the unavailability of detailed reliable data, while the sophisticated modelling of the various forest types and management regimes would complicate the analysis without increasing its accuracy proportionally. The original form of the SOFI can be used in analyses that include economic comparisons across forest types or forests of various geographical regions. Its major benefit is its slim construction, for which comparable input data can be easily obtained, and the same methodology can be applied on a large geographical scale. It can be further refined for specific purposes. Examples for such modifications have been presented in this paper. However, further elaboration requires additional input data, which eliminates the SOFI's advantages.

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Bulletin of Forest Science (Erdészettudományi Közlemények) is a journal supported by the NARIC Forest Research Institute and the Faculty of Forestry of the University of Sopron. The papers are in Hungarian, with English summaries. The recent issue (Vol. 10, 2020) contains the following papers. The full papers can be found and downloaded in *Pdf* format from the journal's webpage (www.erdtudkoz.hu).

Vol. 10, Nr. 1, 2020

Klára CSEKE, Zoltán Attila KÖBÖLKUTI, Attila BENKE, Andrea RUMI, Mátyás BÁDER, Attila BOROVICS and Róbert NÉMETH:

Allelic variation in candidate genes associated with wood properties of cultivated poplars...5–16

Abstract - Poplars represent high economic value. The aim of the present study was to initiate a research methodology that at first identifies candidate genes encoding enzymes with wood property phenotypic traits, towards the aim of developing a genomics-based breeding technology. As a first step, primer pairs were designed on the coding region of 24 candidate genes. 55 primer pairs were tested with 47.27% success rate. In the next phase, eight enzymes were selected for further analysis on 23 genotypes containing seven different poplar species and 11 hybrids. One group of the analyzed enzymes is involved in the lignification process (COMT, CCoAOMT, SAMS), another group (Kt, ptk2, SKOR) holds a key function in K⁺-dependent xylogenesis, while two more enzymes (endo-1,4- β -xylanase, Araf-ase) have a role in determining microfibril angle. 13 different marker regions were successfully amplified, and 188 sequences were analyzed, altogether resulting in 90 SNPs. The number of polymorphic sites, nucleotide diversity, the number of insertions/deletions, the minimum number of recombination events and the linkage disequilibrium were calculated, while the character of SNPs and conserved DNA regions were identified as well. Potential application fields are discussed along with the presented results.

<https://doi.org/10.17164/EK.2020.001>

Zoltán BÖRCSÖK and Zoltán PÁSZTORY:

Changes in the heat conducting properties of wood materials as a result of thermal treatment...17–27

Abstract - The aim of the research is to detect correlations between the heat treatment of wood at different durations and some of its physical properties and thermal conductivity. During the experiments, spruce (*Picea abies*), Pannonia poplar (*Populus ×euramericana* cv. Pannonia) and rubber wood (*Hevea brasiliensis*) were subjected to heat treatment at 180°C

for 15, 25 and 35 hours. Measurements confirmed that the equilibrium moisture content, the density and the thermal conductivity of the specimens made of heat-treated material were lower than those of the untreated specimens. The average equilibrium moisture content decreased from an initial value of around 12% to around 6% during the treatments in case of all three tree species. The decrease in density after 15, 25 and 35 hours of treatment was 9.1, 12.1 and 13.4% for poplar, 5.2, 7.6 and 8.7% for spruce and 3.5, 5.1% and 7.1% for rubber wood, respectively. The decrease in density after 15, 25 and 35 hours of treatment was 17.0, 24.2, 25.2% for poplar, 8.5, 11.6, 19.2% for spruce and 3.6, 4.1, and 8.0% for rubber wood, respectively. Literature data supports that heat treatment decreases the equilibrium wood moisture and density of the wood which explains the lower thermal conductivity compared to the control sample made from the same raw material.

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Zoltán BÖRCSÖK and Zoltán PÁSZTORY:

Improving the properties of bark based insulation panels...29–39

Abstract - Several studies have investigated natural-based insulation materials, including bark. The physical and mechanical properties of the bark panels are worse than those of wood panels. The aims of this study were to manufacture an insulation panel from Pannónia poplar bark and investigate the reinforcement possibilities with short glass fiber, overlaying fibreglass mesh, fibreglass mat and fibreglass woven fabric and two types of paper, as well as inner glass fiber mesh. Further, we tried to improve the thermal conductivity of the panels by heat treating the bark particles. We studied their physical and mechanical properties and thermal conductivity. The target density was 350 kg/m³, the thermal conductivity of the panels ranged from 0.067 to 0.078 W/m·K. The reinforcement slightly decreased thermal conductivity and significantly increased mechanical properties. Thermal conductivity is determined by density. The heat pre-treatment of the raw material slightly decreased the thermal conductivity.

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Bence BOLLA and András SZABÓ:

Early results of the NARIC-FRI hydrological and meteorological monitoring system...41–54

Abstract - The growing extremities of our changing climate has its effects on agriculture, on horticulture and on everyday forestry activities as well. Establishing and maintaining a meteorological monitoring system which measure and collecting data in highly forested areas are the most suitable ways to monitor and keep track of meteorological extremities affecting forests. With the continuous intention to achieve nation-wide coverage, the Forest Research Institute operates 18 GPRS meteorological stations in Hungary. Through analysis of the collected data, we concluded that the meteorological extremities occur at uncommon dates at different points of the country.

The results of the groundwater monitoring system, which is operating alongside the meteorological monitoring, also show significant differences between the hydrological processes of the examined study sites.

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Norbert FRANK and Béla LETT:

Quo vadis forest reproductive material production? (Forest reproductive materials production after the 2nd World War)...55–66

Abstract - After World War II, the forest reproductive material sector was radically reorganized based on central policy instructions, especially in the management and ownership structure. Privately owned forest nurseries have been replaced by small local nurseries of state forest companies, which have produced the necessary reproductive materials next to reforestation and afforestation projects. The Central Instruction for Seedling Production, issued in 1955, already set the goals of rapidly improving quality seedling production, for which purpose the seedling production was concentrated. During the period of 1949–1979, the number of registered forest nurseries decreased from 1,126 to 566. The aim of our study is to analyze the process and trend of seedling production and use over this period and to analyze the data and changes of certain tree species and groups of tree species. Overall, the number of nurseries was significantly decreased during the period under review, the volume of seedlings produced and proportion of tree species were also changed, unevenly over time. In the centrally planned economy period we examined, the difference in the amount of seedlings produced and used per year averaged 66.89 million; the production surplus reached its lowest level in 1975 (21.1 million). The average gross nursery area of the studied period was 3,396 hectares but the average area from 5.55–2.26 hectares in 1954–1959 increased to 5.45 hectares by the end of the period (1979), thus it exceeded the average area typical for the period (3.5 hectares) by 1.95 hectares.

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Vivien SASS, Péter ÓDOR and András BIDLÓ:

The effects of different forestry treatments on litter conditions in an oak-hornbeam forest...69–82

Abstract - The long-term effects of different forestry treatments (clear-cutting, preparation cutting, retention tree groups, gap-cutting) on litter conditions were studied in the framework of the "Pilis Forestry Systems Experiment". During the four-year period described in this publication, the average litter features of the closed control forest area remained unchanged, however, the treatments significantly influenced all the studied litter-variable (quantity, moisture, pH). Litter quantity was the highest in retention tree groups, although this area was the driest. The lowest quantity of litter was measured in clear-cutting. The treatments had the highest effect on the acidity/alkalinity: pH increased in case of clear-cuttings and a less extent gap-cuttings, caused by the increased herbaceous understory cover. We can conclude that moderate partial cutting (preparation cutting) did not change the litter conditions, retention tree groups can buffer the extreme effect of clear-cuttings, and gaps only slightly modify the litter conditions compared to the clearcuts. These results show that continuous cover forestry maintain more favorable litter conditions than rotation forestry systems.

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Géza RIPKA:

Eriophoid mites (Acari: Eriophyoidea) of woody forest plants...83–95

Abstract - Eriophyoid mites are the smallest arthropods living on vascular plants. Representatives of this superfamily can be found on the shoots, foliage, flowers and fruits of herbaceous and woody plants. The majority of the host plants are woody species. An overview is given on the eriophyoid mite fauna of woody forest plants. In Hungary, out of the 238 eriophyoid species 45 are non-indigenous. The most species is recorded from the families Rosaceae and Salicaceae.

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András KOLTAY, Ágnes FÜRJES-MIKÓ, Imola TENORIO-BAIGORRIA, Csaba Béla EÖTVÖS and László HORVÁTH:

Health condition investigation of forests in Kaszó-Life project...97–108

Abstract - The „KASZÓ-LIFE” programme, which was developed between 2014 and 2018, aimed the restoration and improvement of the growing conditions of the Common alder (*Alnus glutinosa*) and the Pedunculate oak (*Quercus robur*) tree estimate. With the help of water retention facilities in the area, the reduction of the subsidence of groundwater was attempted, as well as the improvement of the water balance of the forest's soil, and thus the health of the forests. Sampling areas have been designated in order to monitor the health conditions of the forests where the tree examination is carried out twice a year. Comparing the health data along with the results of groundwater, precipitation and meteorological measurements, we may come to a conclusion in the future concerning the forthcoming changes exhibited in the forests. Examinations so far have shown that both in the case of oaks and alders, from 2017 there was a slight improvement comparing to the control areas. However, due to the relative shortness of the period, this does not yet clearly indicate the success of the programme, which must be supported by many years of test results.

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László BALI, Katalin TUBA and Csaba SZINETÁR:

Arachnological survey of the Roth selection forest...109–124

Abstract - During our research, we surveyed the ground-dwelling spider fauna of the Roth selection forest (Sopron 182B) between April and July of 2020. Five distinct parts of the forest were investigated: pole stand (R, d=10–20 cm), high forests (Sz, d=20–50 cm), older high forests (L, d>50 cm), open/gap habitat (Ny) and stand mixed with spruce (F) patch, with the addition of three control (K) trappings. We also conducted stand structural surveys. We collected 3515 specimens of 69 species belonging to 21 families. *Linyphiidae* was the most species-rich family, while *Pardosa alacris* was the most abundant species. Diversity of the spider community was relatively high. Both the guild structure and the similarity indices showed disparities between the studied sites, however, there were no significant differences. To sum up, the ground-dwelling spider community of the Roth selection forest proved to be somewhat richer than that of the control sites.

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Ákos PALKÓ, Gábor ÓNODI, Tamás RÉDEI and Dániel WINKLER:

Soil eco-faunistic study in lowland relict steppe oak forests and in replacement non-native tree plantations...125–139

Abstract - The aim of the present study was to investigate the soil Collembola communities in the relict closed lowland steppe oak forests in the Kiskunság. Further goal was to carry out comparative analyses of Collembola community diversity and abundance between the autochton oak forests and the replacement allochton plantations of non-native tree species (hybrid poplar, black pine, black locust). Soil samples were taken from the above-mentioned four forest habitats in three replicates. A total of 3,033 specimens belonging to 56 Collembola species were collected and identified. Species richness was the highest (47) in the autochton steppe oak forests. In comparison, the number of species was less than a half in the hybrid poplar (19), black pine (22) and black locust (23) plantations. Regarding the relationships between the measured soil parameters and Collembola communities, positive correlations were found between the C/N ratio and Collembolan abundance ($r=0.71$; $F=10.44$, $p<0.05$) and between soil organic matter content and Collembola diversity ($r=0.61$, $F=5.98$, $p<0.05$). The canonical correspondence analysis (CCA) well separated the steppe oak forests and the non-native plantations along the axis mostly determined by soil pH and carbon content.

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